3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of fluorine, hydrogen fluoride, and fluorides. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

Fluorine is a gaseous element that occurs only in very low concentrations in the environment in the absence of anthropogenic sources (see Chapter 6 for further discussion). Because it is strongly electronegative, it is rarely found in the environment in the elemental state, nor is it likely to be found in the environment near toxic waste sites as molecular fluorine.

Hydrogen fluoride is also a gas and it is very water soluble. When hydrogen fluoride is dissolved in water, it is called hydrofluoric acid. Hydrogen fluoride is very water-soluble and dissolves readily in any water present in the air or other media. Although hydrofluoric acid is very corrosive and can etch glass, it is a weak acid, meaning that it can be present in water as an undissociated molecule. However, in dilute solutions, it is almost completely ionized; salts are formed if cations are available. Due to formation of complexes, very concentrated solutions of hydrofluoric acid are also largely ionic in nature. Therefore, a hydrogen fluoride or hydrofluoric acid spill would result in contamination with fluoride ion, but hydrogen fluoride or hydrofluoric acid would not be of concern outside the immediate vicinity of the spill. However, while members of the public are only likely to come into contact with fluoride contamination, clean-up workers could be exposed to hydrogen fluoride/hydrofluoric acid. In this profile, hydrogen fluoride is used to refer to the gas, while hydrofluoric acid is used to refer to the liquid form. When both forms are included, the term hydrogen fluoride is used.

The term fluoride properly refers to numerous natural and synthesized compounds that are derived from hydrofluoric acid. This class of chemicals is commonly referred to as fluorides. Some of these compounds, such as oxygen difluoride, are very reactive and highly toxic. Because of their reactivity, these compounds would not migrate unchanged from a hazardous waste site. Fluoride salts, such as sodium fluoride and calcium fluoride, are much less reactive and much less toxic. Since the fluoride ion is the toxicologically active agent, and discussion of water fluoridation uses the term fluoride, the term fluoride is used generically in this profile to refer to toxicology of fluoride salts. Because numerous

different fluoride compounds exist naturally in the environment and have varying chemical properties, the term fluorides is used in the discussion of environmental media. Most of the available literature on fluoride toxicity concerns sodium fluoride. Additional toxicity literature is available on some other forms of fluoride, such as stannous fluoride. Other forms of fluoride are discussed only if exposure is likely to occur at a hazardous waste site. (Such exposure to stannous fluoride is not likely.) Wherever the form of fluoride exposure is known, that salt is identified in the profile.

Limited information also exists concerning occupational exposure to the mineral cryolite (Na_3AlF_6), sometimes with concomitant exposure to hydrogen fluoride. Because these exposures usually involve exposure to both hydrogen fluoride and cryolite, sometimes along with exposure to other fluoride dusts, they are discussed separately in the profile.

This profile will discuss data, or the absence of data, concerning the toxicity of inorganic compounds of fluorine that people could be exposed to at a hazardous waste site. Exposure and toxicity are discussed separately for fluorine, hydrogen fluoride/hydrofluoric acid, and fluoride. Toxic effects of occupational exposure in aluminum reduction plants, where exposure to hydrogen fluoride, fluoride dusts, and cryolite all occur, are also discussed separately. Because the toxic effects of fluorine are largely due to the action of the fluorine molecule on the respiratory tract or other exposed surfaces, fluorine exposure is reported as exposure to a level of diatomic fluorine. By contrast, systemic effects of hydrogen fluoride are due to the fluoride ion, so concentrations of hydrogen fluoride are converted to fluoride equivalents. All doses of fluoride are reported as amount of fluoride ion.

The primary routes and durations of concern vary with the different fluorine compounds. In general, the more soluble the fluoride is, the more that can be absorbed by oral ingestion, and the more toxic it is. The primary exposure routes and duration for hydrofluoric acid are the inhalation or dermal routes, related to acute occupational exposure, while the primary exposure route and duration for fluoride is chronic exposure to fluoride in the drinking water, food, and fluoride-containing dental products. Therefore, most of the information for the inhalation and dermal routes comes from studies of acute exposure to fluorine or hydrofluoric acid, while most of the information regarding the oral route is based on sodium fluoride. The toxicity following inhalation or dermal exposure to other inorganic fluorine compounds differs from that of hydrofluoric acid. Similarly, oral exposure to various fluorides other than sodium fluoride may result in different toxic effects.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of fluorine, hydrogen fluoride, and fluorides are indicated in Table 3-3 and Figure 3-3. Because cancer effects could occur at lower exposure levels, Figure 3-3 also shows a range for the upper boundary of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

Inhalation exposure most commonly occurs in an occupational setting. As discussed above, most of the available information concerning toxic effects of fluorine and its compounds following inhalation exposure comes from studies of exposure to hydrogen fluoride or hydrofluoric acid. There are also a limited number of useful studies concerning inhalation exposure to fluorine or particulates of inorganic fluoride compounds. However, no animal studies were located regarding toxic effects of exposure to the particulate fluoride compounds. Toxic effects of hydrogen fluoride are discussed in all of the following sections. Where toxicity data exist for fluorine or fluoride, these substances are also discussed.

Fluorine gas is extremely irritating. The primary health effects of acute fluorine inhalation are nasal and eye irritation (at low levels), and death due to pulmonary edema (at high levels). In animals, renal and hepatic damage have also been observed.

Acute inhalation of hydrogen fluoride following facial splashes with hydrofluoric acid can cause bronchiolar ulceration, pulmonary hemorrhage and edema, and death. In addition, renal and hepatic damage have been observed in animal studies. Many of the human studies regarding inhalation of hydrogen fluoride fumes also involved dermal exposure; in such cases, it is difficult to determine which effects are specific to the inhalation route. However, the respiratory effects of hydrogen fluoride appear to be inhalation-specific, because they have not been reported in cases where there was clearly no inhalation exposure. The effects of combined inhalation and dermal exposure to hydrofluoric acid are also discussed in Section 3.2.3.

The major health effect of chronic inhalation exposure to fluoride is skeletal fluorosis, which has been reported in cases of exposure to fluoride dusts and hydrogen fluoride, either individually or in combination.

3.2.1.1 Death

Both fluorine and hydrogen fluoride can cause lethal pulmonary edema, although cardiac effects also contribute to the toxicity of hydrogen fluoride. The reported LC_{50} values for hydrogen fluoride in rats for a given duration are generally at least 3.5 times higher than the value for fluorine (as diatomic fluorine) in rats for the same duration. Although strain differences could account for some of this difference, the LC_{50} values of hydrogen fluoride in Crl:CD®BR and Wistar-derived rats were very similar.

Fluorine. No information was located on death in humans caused by fluorine. Fluorine toxicity has been investigated in Osborne-Mendel rats, Swiss-Webster mice, New England guinea pigs, and New Zealand rabbits (Keplinger and Suissa 1968). Similar values for the LC_{50} were calculated for the different species. In the rats, the LC_{50} values for exposures of 5, 15, 30, and 60 minutes were 700, 390, 270, and 185 ppm, respectively. At concentrations near the LC_{50}, few signs of intoxication were observed immediately after exposure, except for irritation of the eyes and nose. Several hours after exposure, the animals exhibited lethargy, dyspnea, and general weakness. Except at concentrations above the LC_{90}, death generally occurred 12–18 hours after exposure. Animals that survived for 48 hours generally survived for the duration of the observation period. Loss of body weight was also observed, but was considered nonspecific and was attributed to anorexia.

Toxic effects of inhalation exposure to fluorine and hydrogen fluoride were compared in rats, mice, rabbits, and guinea pigs (Stokinger 1949). Lethal doses from fluorine exposure determined by this group are about 3–4 times those determined by Keplinger and Suissa (1968), but quantitative exposure level data from these experiments are not reliable due to technical problems in monitoring fluorine gas levels. However, qualitative results from these experiments are useful. These experiments also found that fluorine was more toxic than hydrogen fluoride.

There are some indications that preexposure to low levels of fluorine may provide resistance to lethal effects of fluorine. Increased survival times were seen in New Zealand rabbits when challenged 48 hours after a preexposure regimen (Keplinger 1969). For example, 4 weeks of exposure to 50 ppm for 30 minutes once/week increased the survival time following a 30-minute challenge with 400 ppm from a maximum 18–48 hours. Small increases in the LC_{50} were observed when mice were preexposed 4 times in 7 days to 25 ppm for 15 minutes/exposure, followed by a challenge exposure 24–168 hours later. No mechanism for the possible tolerance was suggested.

Repeated exposures of rats, mice, guinea pigs, and rabbits to 0.5, 2, 5, or 18 ppm fluorine were conducted for up to 178 hours over 35 days (Stokinger 1949). The exposure regimen was not stated, but appears to be 6 hours/day, 6 days/week. The exposure levels at these lower concentrations were considered fairly reliable. Guinea pigs and rats were less sensitive to lethal effects than were rabbits or dogs. All of the rabbits and dogs exposed to 5 ppm and mice exposed to 18 ppm died, while only half of the rats and guinea pigs exposed to 18 ppm died. Most animals exposed to 2 ppm survived.

Hydrogen Fluoride. Acute inhalation of hydrogen fluoride fumes in combination with dermal exposure to hydrofluoric acid has been reported to cause death in humans. Actual exposure concentrations are not known in any of these cases. Death was generally due to pulmonary edema (resulting from irritation and

constriction of the airways), or cardiac arrhythmias with pronounced hyperkalemia, hypocalcemia, and hypomagnesemia.

The death of a chemist who sustained first- and second-degree burns of the face, hands, and arms when a vat containing hydrofluoric acid accidentally ruptured has been described (Kleinfeld 1965). This 29-year-old male died 10 hours after admission to the hospital. Postmortem examination revealed severe tracheobronchitis and hemorrhagic pulmonary edema. A petroleum refinery worker was splashed in the face with 100% anhydrous hydrofluoric acid (Tepperman 1980). The absorption of fluoride produced acute systemic fluoride poisoning with profound hypocalcemia and hypomagnesemia and cardiac arrhythmias. The patient died <24 hours after exposure; autopsy revealed pulmonary edema. A young woman splashed in the face with hydrofluoric acid died of respiratory insufficiency a few hours after exposure (Chela et al. 1989). The autopsy revealed severe burns of the skin and lungs, with hemorrhagic pulmonary edema produced by hydrofluoric acid and its vapor.

The lethal concentration of hydrogen fluoride has been investigated in rats, mice, and guinea pigs. It appears that mice are more sensitive to the acute effects of hydrogen fluoride than rats, and rats are more sensitive than guinea pigs. The 15-minute LC_{50} values for hydrogen fluoride were 4,327 ppm fluoride for guinea pigs and 2,555 ppm fluoride for Wistar-derived rats (Rosenholtz et al. 1963). The 60-minute LC_{50} values for hydrogen fluoride were 325 ppm fluoride in ICR-derived mice (Wohlslagel et al. 1976), 1,325 ppm fluoride in Sprague-Dawley-derived rats (Wohlslagel et al. 1976), and 1,242 ppm fluoride in Wistar-derived rats (Rosenholtz et al. 1963).

The LC_{50} values reported by Haskell Laboratory (1988) for Crl:CD®BR rats were much higher than the values reported by the above investigators, although the size of the discrepancy decreased with longer exposure durations. For example, the 15-minute LC_{50} was reported as 6,620 ppm, while the 60-minute LC_{50} was 1,610 ppm. Although the concentration of hydrogen fluoride that produced death was reported to be lower when it was administered to rats in humid air (Haskell Laboratory 1988), the method for measuring fluoride in humid air may not have given accurate results. This limitation was recognized by the authors, who stated that the collection efficiency of the sampling train for aerosols was not evaluated.

Longer-term effects of hydrogen fluoride were investigated by exposing various species to 8.2 or 31 ppm fluoride as hydrogen fluoride for 6 hours/day, 6 days/week for 5 weeks (Stokinger 1949). Humidity was 47–97% at the lower concentration, and 48–66% at the higher concentration. Marked species differences were observed. All rats and mice exposed to 31 ppm died, but no guinea pigs, rabbits, or dogs exposed at this level died. No animal of any species died following exposure to 8.2 ppm. In an experiment where five rabbits, three guinea pigs, and two Rhesus monkeys were exposed to 18 ppm for 6–7 hours/day, 5 days/week for 50 days (309 hours total), the only deaths observed were two guinea pigs (Machle and

Kitzmiller 1935). Exposure of one of these animals stopped after 134 hours of exposure, and exposure of the other one stopped after 160 hours, when marked weight loss was observed. Nevertheless, the animals died about 2 weeks later.

The LC_{50} values for each species and duration category of exposure to fluorine are recorded in Table 3-1 and plotted in Figure 3-1. The LC_{50} values for each species and duration category of exposure to hydrogen fluoride are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.1.2 Systemic Effects

The predominant systemic effects of acute inhalation exposure to fluorine or hydrogen fluoride are respiratory, nasal, and ocular irritation.

Kidney and liver necrosis have also been observed in animals. No data were located regarding chronic inhalation exposure to fluorine. Most of the data that were located regarding systemic effects of chronic inhalation exposure are from occupational exposure to fluoride dusts, sometimes in combination with hydrogen fluoride. In these cases, the predominant systemic effect is skeletal fluorosis. Preexisting conditions were generally not determined in the occupational or case studies, and levels of exposure and exposure durations were often approximations.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category of exposure to fluorine are recorded in Table 3-1 and plotted in Figure 3-1. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category of exposure to hydrogen fluoride are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. Both fluorine and hydrogen fluoride irritate the respiratory tract and can cause hemorrhaging in a duration- and concentration-dependent manner. Nasal irritation is discussed under dermal exposure (Section 3.2.3) because it is caused by direct contact with the gases.

Fluorine. Limited data are available regarding respiratory effects of fluorine on humans. Five volunteers (19–50 years of age; gender not specified) were exposed to fluorine through a face mask that covered the eyes and nose but not the mouth (Keplinger and Suissa 1968). A concentration of 10 ppm was not irritating to the respiratory tract for at least 15 minutes. Slight nasal irritation was reported following a 3-minute exposure to 50 ppm, and exposure to 100 ppm for 0.5 or 1 minute was very irritating to the nose. Intermittent inhalation (3–5 minute exposure every 15 minutes for 2–3 hours) of 23 ppm did not cause respiratory difficulty.

Table 3-1. Levels of Significant Exposure to Fluorine - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
ACUTE EXPOSURE							
Death							
1	Rat (Osborne-Mendel)	1d 5-60min/d				700 (5-minute LC_{50})	Keplinger and Suissa 1968 fluorine
						390 (15-minute LC_{50})	
						270 (30-minute LC_{50})	
						185 (60-minute LC_{50})	
2	Mouse (Swiss-Webster)	1d 15-60min/d				600 (5-minute LC_{50})	Keplinger and Suissa 1968 fluorine
						375 (15-minute LC_{50})	
						225 (30-minute LC_{50})	
						150 (60-minute LC_{50})	
3	Gn Pig (New England)	1d 15-60min/d				395 (15-minute LC_{50})	Keplinger and Suissa 1968 fluorine
						170 (60-minute LC_{50})	
4	Rabbit (New Zealand)	1d 5-30min/d				820 (5-minute LC_{50})	Keplinger and Suissa 1968 fluorine
						270 (30-minute LC_{50})	
Systemic							
5	Human	1d 1min/d	Resp		67 (nasal irritation)		Keplinger and Suissa 1968 fluorine
6	Human	1d 3min/d	Resp	10	50 (slight nasal irritation)		Keplinger and Suissa 1968 fluorine

Table 3-1. Levels of Significant Exposure to Fluorine - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
7	Human	1d 5min/d	Resp	10			Keplinger and Suissa 1968 fluorine
8	Human	1d 3-5min every 15 min for 2-3 hr	Resp	23			Keplinger and Suissa 1968 fluorine
9	Human	1d 15 min	Resp	10[b]			Keplinger and Suissa 1968 fluorine
10	Human	1d 0.5min/d	Resp		100 (nasal irritation)		Keplinger and Suissa 1968 fluorine
11	Rat (Osborne-Mendel)	1d 5min/d	Resp	88	175 (dyspnea; mild lung congestion)	350 (irritation)	Keplinger and Suissa 1968 fluorine
12	Rat (Osborne-Mendel)	1d 15min/d	Resp	49	98 (very mild lung congestion)	195 (irritation)	Keplinger and Suissa 1968 fluorine
13	Rat (Osborne-Mendel)	1d 30min/d	Resp	35	70 (very mild lung congestion)	140 (nasal irritation)	Keplinger and Suissa 1968 fluorine
14	Rat (Osborne-Mendel)	1d 60min/d	Resp	28	47 (very mild lung congestion)	93 (nasal irritation)	Keplinger and Suissa 1968 fluorine

Table 3-1. Levels of Significant Exposure to Fluorine - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)	Reference Chemical Form
15	Mouse (Swiss-Webster)	1d 5min/d	Resp	79	174	(dyspnea; mild lung congestion, slight alveolar necrosis)		Keplinger and Suissa 1968 fluorine
			Hepatic	174	195	(necrosis and cloudy swelling)		
			Renal	79	114	(necrosis)		
16	Mouse (Swiss-Webster)	1d 15min/d	Resp	65	87 188	(very mild lung congestion) (irritation)		Keplinger and Suissa 1968 fluorine
17	Mouse (Swiss-Webster)	1d 15min/d	Resp	65	82	(alveolar necrosis and hemorrhage)		Keplinger and Suissa 1968 fluorine
			Hepatic	128	144	(coagulation, necrosis, and cloudy swelling)		
			Renal	65	82	(coagulation, necrosis)		
18	Mouse (Swiss-Webster)	1d 30min/d	Resp	51	82	(alveolar necrosis and hemorrhage)		Keplinger and Suissa 1968 fluorine
			Hepatic	82	116	(coagulation, necrosis, and cloudy swelling)		
			Renal	51	82	(coagulation, necrosis)		
19	Mouse (Swiss-Webster)	1d 30min/d	Resp	32 67	67 113	(very mild lung congestion) (irritation)		Keplinger and Suissa 1968 fluorine
20	Mouse (Swiss-Webster)	1d 60min/d	Resp	30 75	50 150	(very mild lung congestion) (nasal irritation)		Keplinger and Suissa 1968 fluorine

Table 3-1. Levels of Significant Exposure to Fluorine - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
21	Mouse (Swiss-Webster)	1d 60min/d	Resp	30	50 (alveolar necrosis and hemorrhage)		Keplinger and Suissa 1968 fluorine
			Hepatic	55	80 (necrosis,and cloudy swelling)		
			Renal	50	55 (necrosis)		
22	Gn Pig (New England)	1d 15min/d	Resp	70	100 (very mild lung congestion) 198 (irritation)		Keplinger and Suissa 1968 fluorine
23	Gn Pig (New England)	1d 60min/d	Resp	73	135 (mild lung congestion, irritation, dyspnea)		Keplinger and Suissa 1968 fluorine
24	Dog (NS)	1d 15min/d	Resp	39	93 (slight lung congestion)		Keplinger and Suissa 1968 fluorine
25	Dog (NS)	1d 60min/d	Resp	68	93 (irritation, cough, slight dynspnea, lung hemorrhage)		Keplinger and Suissa 1968 fluorine
26	Rabbit (New Zealand)	1d 5min/d	Resp	79	134 (slight dyspnea) 410 (irritation)		Keplinger and Suissa 1968 fluorine
27	Rabbit (New Zealand)	1d 30min/d	Resp	32	71 (very mild lung congestion) 135 (irritation)		Keplinger and Suissa 1968 fluorine

INTERMEDIATE EXPOSURE

Table 3-1. Levels of Significant Exposure to Fluorine - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
Death									
28	Rat (NS)	5 wks 6d/wk 6hr/d					18	(24/48 deaths)	Stokinger 1949 fluorine
29	Dog (NS)	5 wks 6d/wk 6hr/d					5	(100% mortality)	Stokinger 1949 fluorine
30	Rabbit (NS)	5 wks 6d/wk 6hr/d					5	(100% mortality)	Stokinger 1949 fluorine
Systemic									
31	Rat (NS)	5 wks 6d/wk 6hr/d	Resp	5	18	(severe pulmonary irritation)			Stokinger 1949 fluorine
			Bd Wt				18	(weight loss)	
32	Dog (NS)	5 wks 6d/wk 6hr/d	Resp	0.5	2	(pulmonary hemorrhage and edema)			Stokinger 1949 fluorine
			Hepatic	5	18	(liver congestion)			
33	Rabbit (NS)	5 wks 6d/wk 6hr/d	Resp	0.5	2	(mild bronchial inflammation)	18	(hemorrhage in the lungs)	Stokinger 1949 fluorine
			Hepatic	2	5	(hyperemia of the liver)			

Table 3-1. Levels of Significant Exposure to Fluorine - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
Reproductive							
34	Rat (NS)	5 wks 6d/wk 6hr/d		5		18 (testicular degeneration)	Stokinger 1949 fluorine

[a]The number corresponds to entries in Figure 3-1.
[b]Used to derive an acute inhalation minimal risk level of 0.01 ppm; concentration adjusted for intermittent exposure (0.25hours/24hours) and divided by an uncertainty factor of 10 for human variability.

d = day(s); Gn Pig = Guinea pig; LC_{50} = lethal concentration, 50% kill; LOAEL; lowest-observed-adverse-effect-level; min = minute(s); NOAEL = no-observed-adverse-effect level; ppm = parts per million; Resp = respiratory

Figure 3-1. Levels of Significant Exposure to Fluorine - Inhalation

Acute (≤14 days)

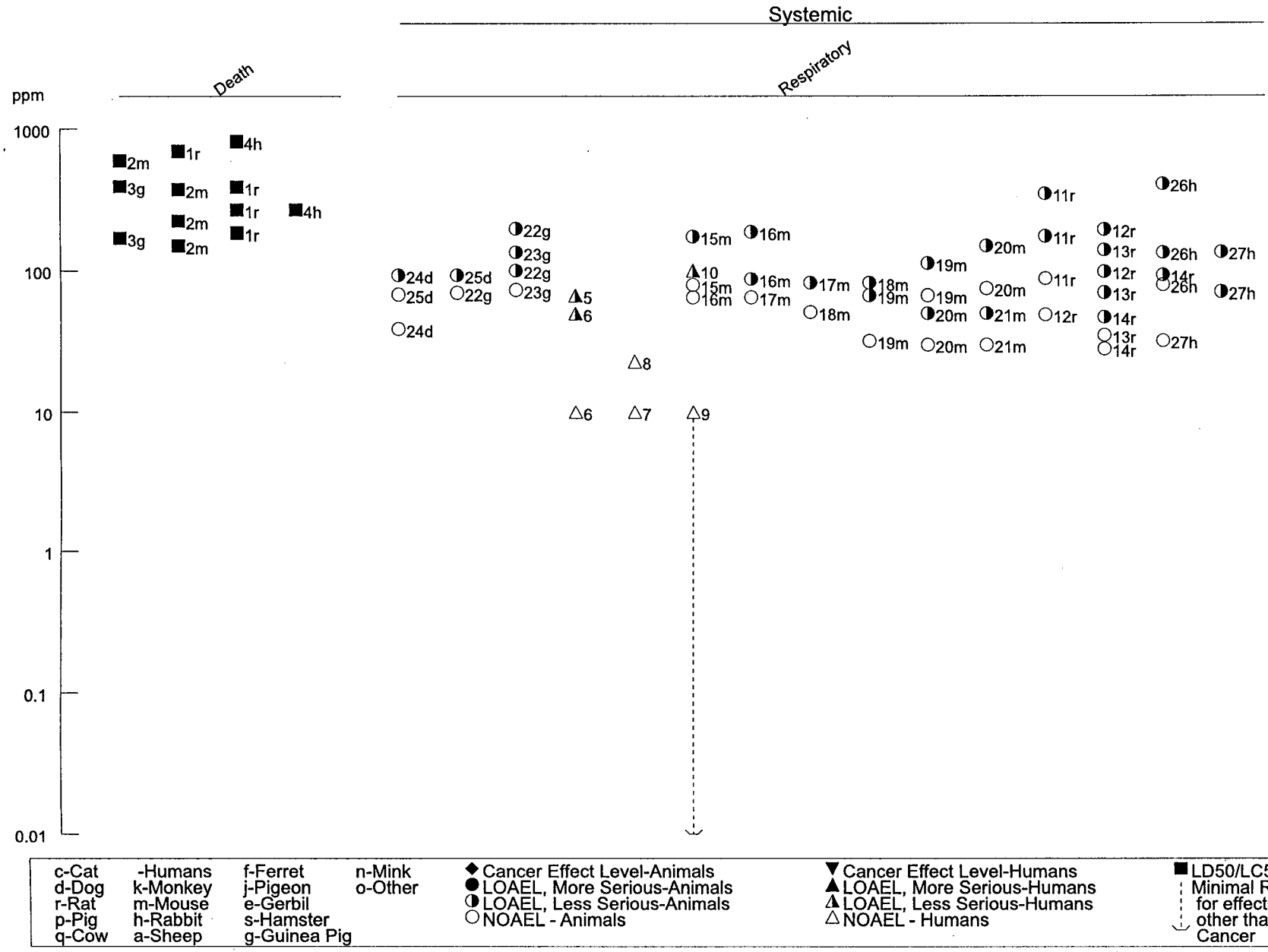
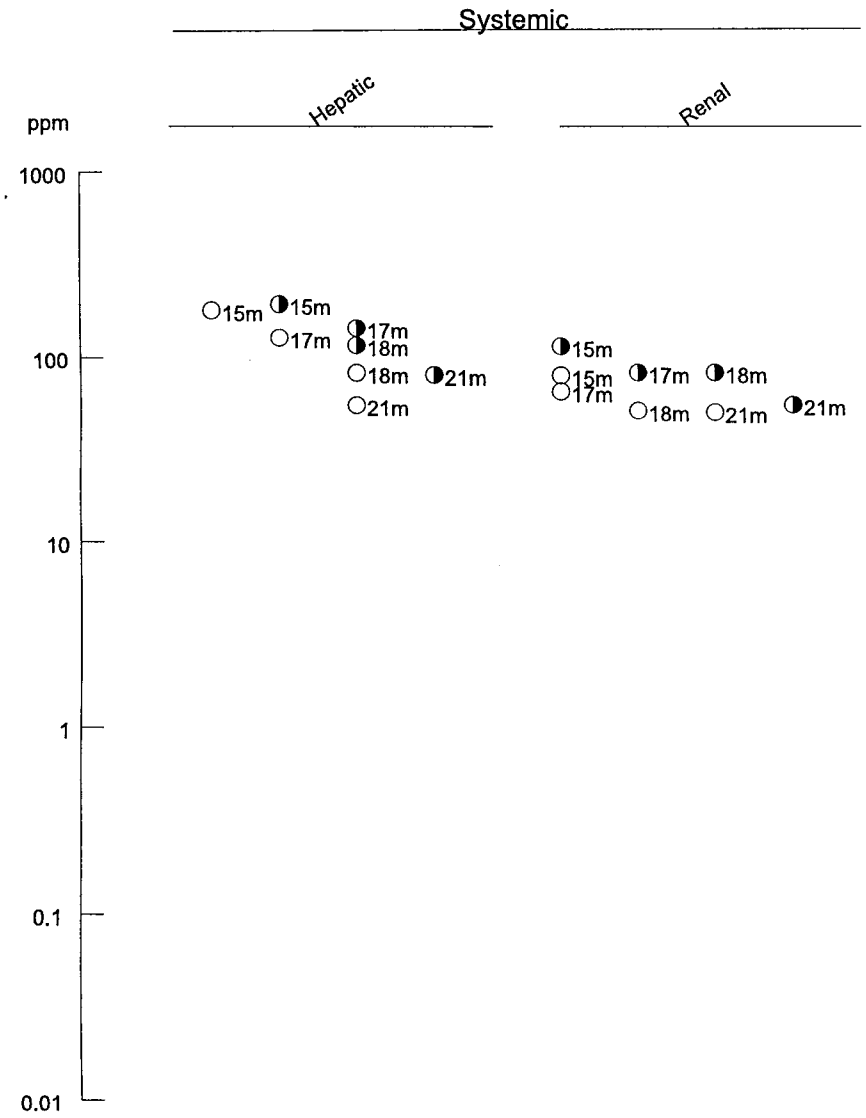


Figure 3-1. Levels of Significant Exposure to Fluorine - Inhalation (*continued*)

Acute (≤14 days)



c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◮ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-1. Levels of Significant Exposure to Fluorine - Inhalation (*continued*)

Intermediate (15-364 days)

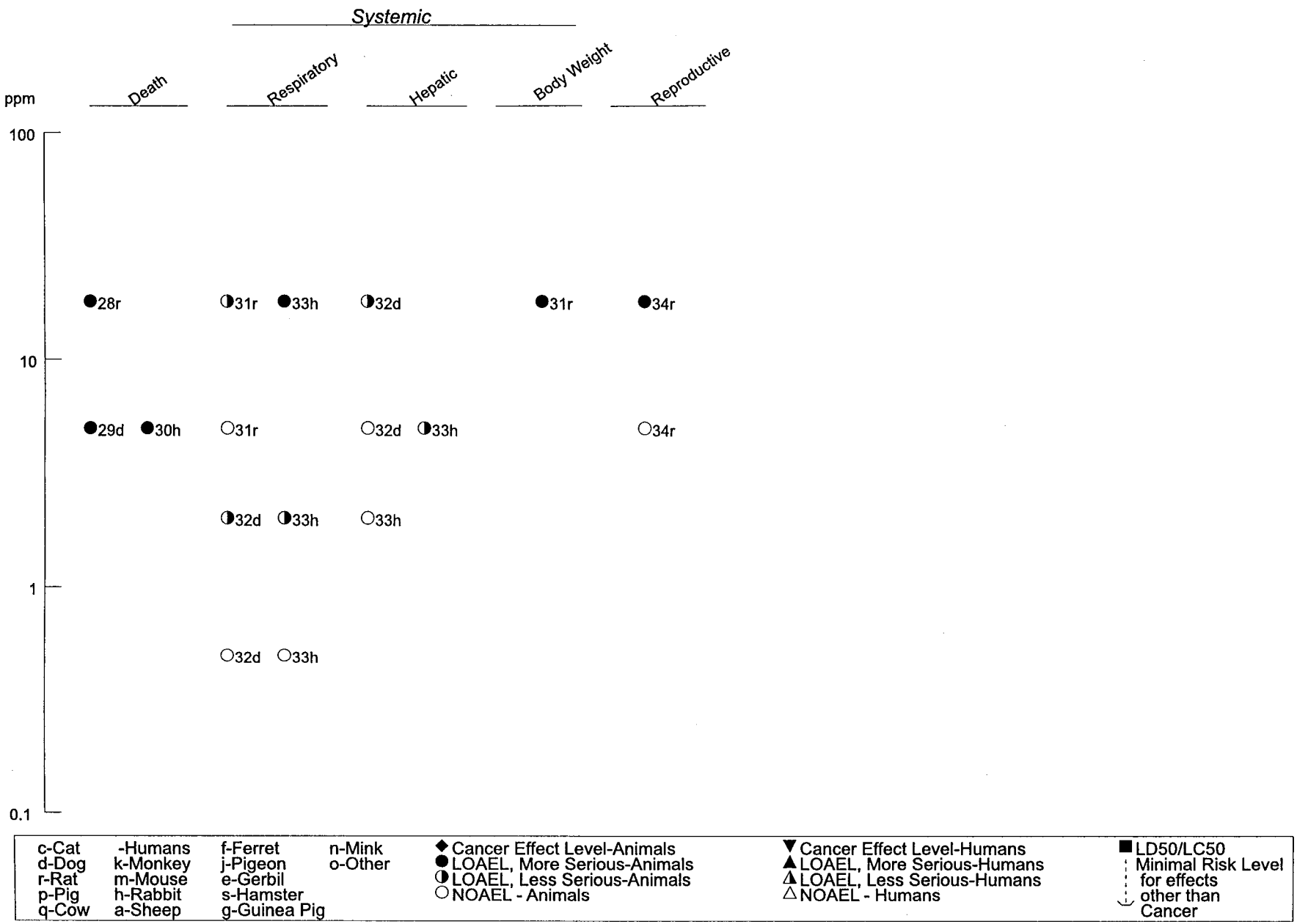


Table 3-2. Levels of Significant Exposure to Hydrogen Fluoride - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form
ACUTE EXPOSURE								
Death								
1	Rat	1 d 5-60 min/d				14,600	(5-minute LC_{50})	Haskell Laboratory 1988 hydrogen fluoride
						6620	(15-minute LC_{50})	
						2890	(30-minute LC_{50})	
						1610	(60-minute LC_{50})	
2	Rat (Wistar)	1 d 5-60 min/day				4722	(5-minute LC_{50})	Rosenholtz et al. 1963 hydrogen fluoride
						2555	(15-minute LC_{50})	
						1940	(30-minute LC_{50})	
						1242	(60-minute LC_{50})	
3	Rat	1 d 60 min/d				1325	(60-minuteLC_{50})	Wohlslagel et al. 1976 hydrogen fluoride
4	Mouse	1 d 60min/d				325	(60-minute LC_{50})	Wohlslagel et al. 1976 hydrogen fluoride
5	Gn Pig (Hartley)	1 d 5-60 min/d				4327	(15-minute LC_{50})	Rosenholtz et al. 1963 hydrogen fluoride

Table 3-2. Levels of Significant Exposure to Hydrogen Fluoride - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
Systemic									
6	Rat (Sprague-Dawley)	2 min	Resp	563	1509	(mucosal necrosis in mid trachea)			Dalbey et al. 1998a, b hydrogen fluoride
			Hemato	4643	8190	(incr RBC, hemoglobin, and hematocrit levels)			
			Hepatic	563	1509	(incr asparate aminotransferase activity)			
7	Rat (Sprague-Dawley)	10 min	Resp	257	902	(minimal midtracheal necrosis)			Dalbey et al. 1998a, b hydrogen fluoride
			Hemato		1676	(incr hemoglobin and hematocrit levels)			
			Hepatic	1676					
8	Rat	1 d 5 min/d	Resp		712	(mild nasal irritation)	2310	(temporary respiratory distress and nasal discharge)	Rosenholtz et al. 1963 hydrogen fluoride
9	Rat	1 d 60min/d	Resp	98[b]	120	(mild nasal irritation)	465	(temporary respiratory distress and nasal discharge)	Rosenholtz et al. 1963 hydrogen fluoride
10	Rat	1 d 15min/d	Resp	292	357	(mild nasal irritation)	1339	(temporary respiratory distress and nasal discharge)	Rosenholtz et al. 1963 hydrogen fluoride

Table 3-2. Levels of Significant Exposure to Hydrogen Fluoride - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
11	Rat (Fischer- 344)	30 min	Resp				1235	(fibrinonecrotic rhinitis in nose breathing rats; tracheal and bronchial necrosis in mouth breathing rats)	Stavert et al. 1991 hydrogen fluoride
			Bd Wt		1235	(10% body weight reduction)			
INTERMEDIATE EXPOSURE									
Death									
12	Rat (NS)	5 wks 6d/wk 6hr/d					31	(death in 29/29)	Stokinger 1949 hydrogen fluoride
13	Mouse (NS)	5 wks 6d/wk 6hr/d					31	(death in 18/18)	Stokinger 1949 hydrogen fluoride
Systemic									
14	Human	15-50 d 6 hr/d	Resp		2.98[c]	(slight nasal irritation)			Largent 1960 hydrogen fluoride
15	Rat (NS)	5 wks 6hr/d	Resp	8.2	31	(pulmonary hemorrhage)			Stokinger 1949 hydrogen fluoride
			Hemato	31					
			Renal	8.2	31	(cortical necrosis)			
16	Dog (NS)	5 wks 6d/wk 6hr/d	Resp		31	(pulmonary hemorrhage)			Stokinger 1949 hydrogen fluoride
			Hemato	31					

Table 3-2. Levels of Significant Exposure to Hydrogen Fluoride - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
17	Rabbit (NS)	5 wks 6d/wk 6hr/d	Resp	8.2	31 (pulmonary hemorrhage)		Stokinger 1949 hydrogen fluoride
			Hemato	31			
Neurological							
18	Rat (albino)	5mo 24hr/d		0.01	0.03 (disturbances in conditioned reflexes; lengthened latent periods)		Sadilova et al. 1965 hydrogen fluoride

[a]The number corresponds to entries in Figure 3-2.
[b]Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.03 ppm; the concentration was adjusted for differences between the rat and human ratio of extrathoracic surface area to minute volume [(0.43 m^3/day/15 cm^2)/(20 m^3/day / 200 cm^2)] and for less than 24 hour exposure (1 hour/24 hours) and divided by an uncertainty factor of 30 (3 for interspecies extrapolation using dosimetric adjustments and 10 for human variability).
[c]Used to derive an intermediate inhalation minimum risk level (MRL) of 0.02 ppm; concentration adjusted for intermittent exposure (6hours/24hours) and divided by an uncertainty factor of 30 (3 for use of a LOAEL of a minimally adverse effect and 10 for human variability).
Bd = body weight; d = day(s); Hemato = hematological; hr = hour(s); incr = increase; LC_{50} = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; min =

Figure 3-2. Levels of Significant Exposure to Hydrogen Fluoride - Inhalation

Acute (≤14 days)

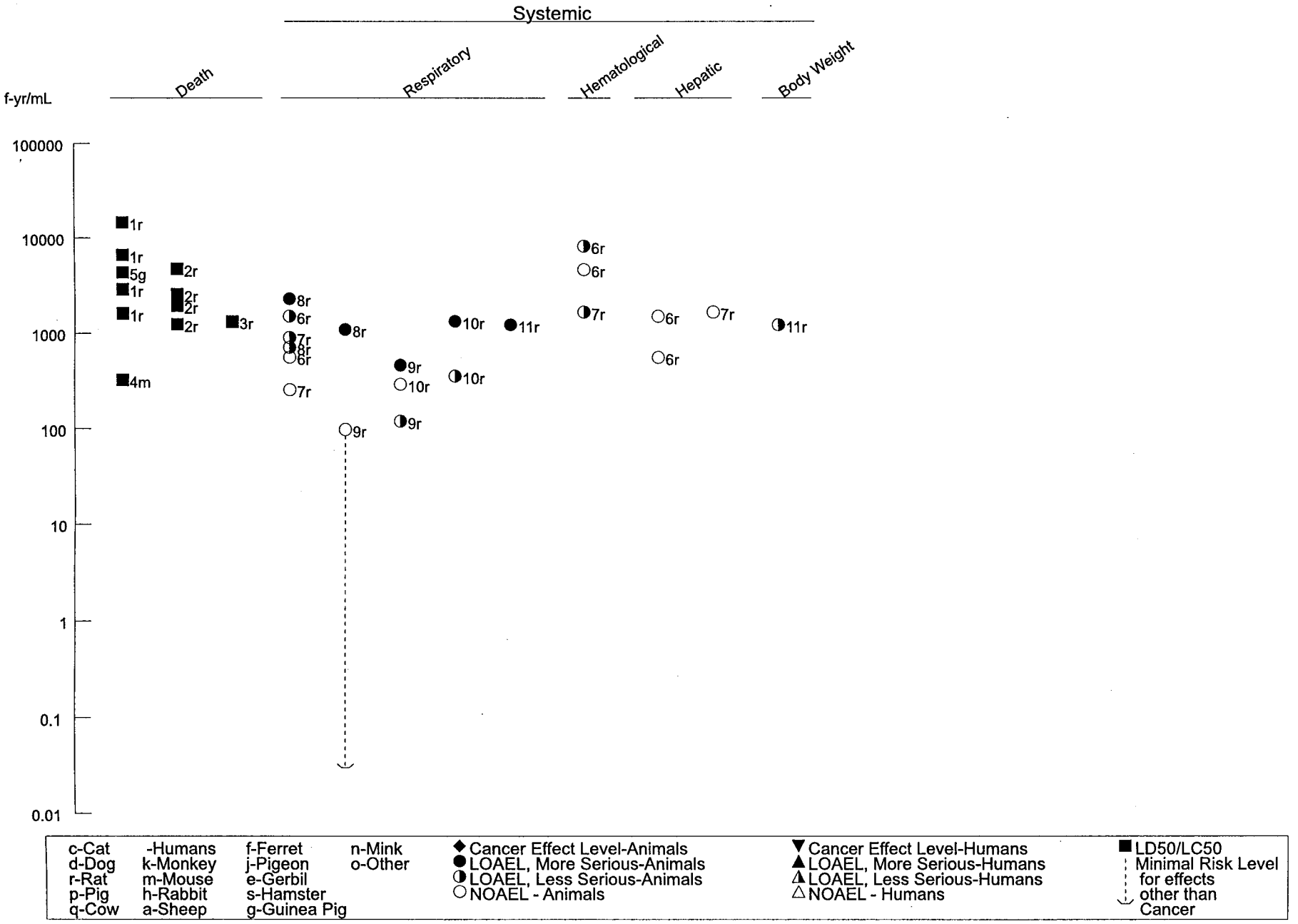
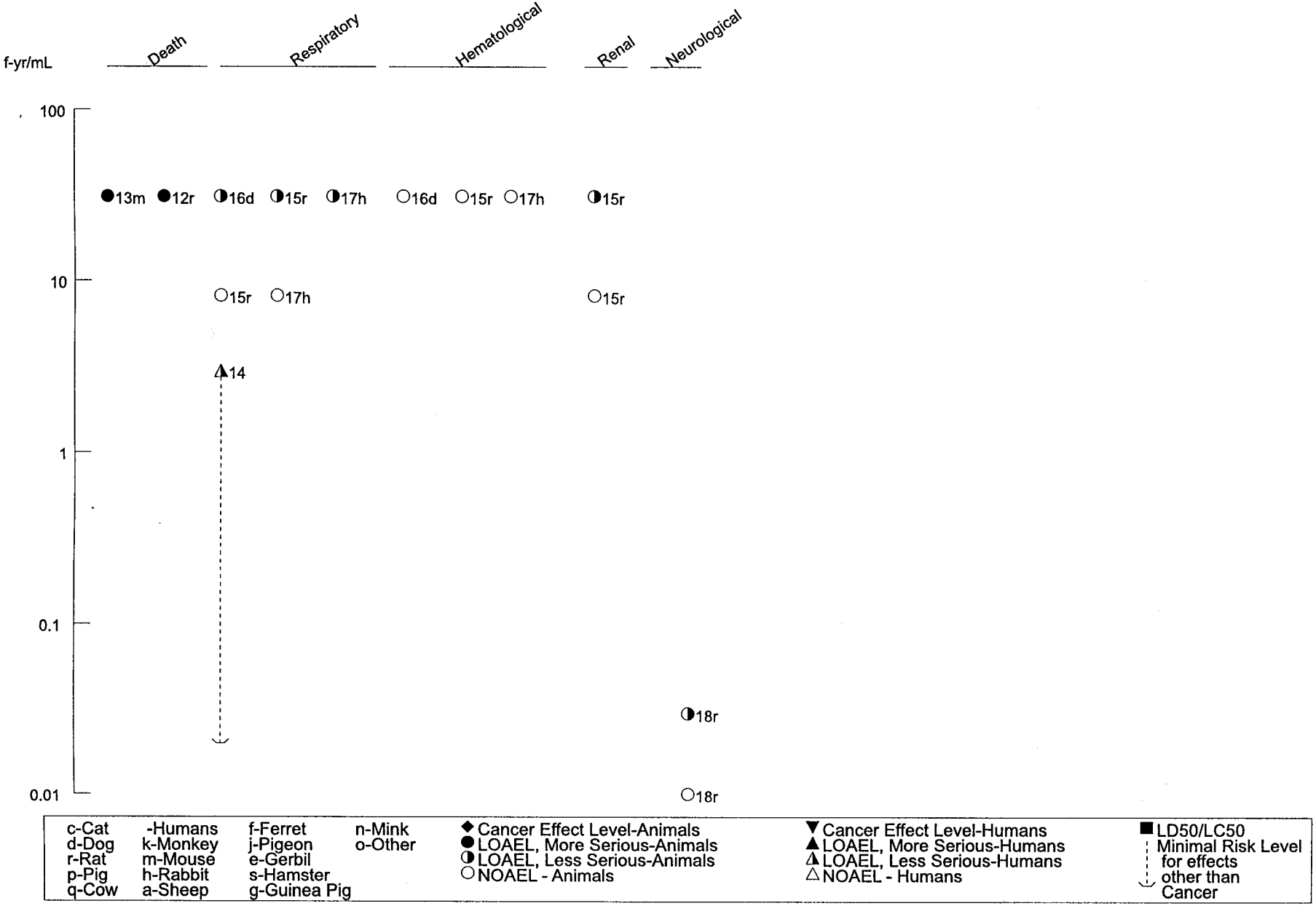


Figure 3-2. Levels of Significant Exposure to Hydrogen Fluoride - Inhalation (*continued*)

Intermediate (15-364 days)



An occupational cohort study comparing the incidence of respiratory complaints by 61 exposed workers with over 2,000 "unexposed" workers found no increase in the exposed group (Lyon 1962). The average fluorine level was 0.9 ppm, and the maximum measured value was 24 ppm. The study author concluded that the workers became "hardened" to the irritating effects of fluorine. The study is limited in that both groups were also exposed to uranium hexafluoride and hydrogen fluoride. The method of measuring respiratory complaints (visits to the plant medical department) was also not very sensitive. However, the observation of tolerance caused by repeated low level exposures is supported by the results from animal studies discussed in Section 3.2.1.1 and later in this section (Keplinger 1969).

Diffuse lung congestion has been reported in rats, mice, guinea pigs, dogs, and rabbits exposed to fluorine for 5–60 minutes (Keplinger and Suissa 1968). The severity was concentrated-related. The adverse effect levels for each exposure duration did not appear to vary across species. The ranges of adverse effect levels for each exposure duration were 174–175 ppm for 5 minutes, 87–100 ppm for 15 minutes, 67–71 ppm for 30 minutes, and 47–135 ppm for 60 minutes. Other respiratory effects that were observed in these animals included dyspnea, irritation, and alveolar necrosis.

In 5-week exposure studies conducted by Stokinger (1949), pulmonary hemorrhage, edema, and bronchial inflammation were reported. These studies found species differences in sensitivity to fluorine-induced respiratory effects. Exposure to 2 ppm, 6 hours/day, 6 days/week for 5 weeks resulted in no effects in rats, pulmonary hemorrhage and edema in dogs, and mild bronchial inflammation in rabbits; respiratory effects (severe pulmonary irritation) were observed in rats exposed to 18 ppm.

Swiss-Webster mice that were preexposed once to 30 ppm fluorine for 60 minutes, and were then exposed to 118–410 ppm fluorine for 15 minutes after an interval of 4–96 hours showed markedly less lung pathology than animals that were not pretreated (Keplinger 1969). At the highest level (410 ppm), exposure 4 hours prior to the challenge reduced the lung pathology from the most severe rating to a rating of normal–mild. Preexposure also reduced the increased lung weight otherwise seen following fluorine exposure. However, a similar preexposure regimen only resulted in slight increases in the LC_{50}, as discussed in Section 3.2.1.1.

Hydrogen Fluoride. Acute inhalation of 122 ppm fluoride as hydrogen fluoride by two male volunteers produced marked respiratory irritation within 1 minute (Machle et al. 1934). Pulmonary edema, pulmonary hemorrhagic edema, and tracheobronchitis have been reported in cases of people being splashed in the face with hydrofluoric acid, where concurrent inhalation and dermal exposure are likely (Chan et al. 1987; Chela et al. 1989; Dieffenbacher and Thompson 1962; Kleinfeld 1965; Tepperman 1980). Exposure concentrations were not known in these cases.

A number of residents of Texas City, Texas, reported respiratory symptoms following the accidental release of hydrogen fluoride. It was estimated that most of the hydrogen fluoride was released in the first 2 hours after the accident, and evacuation of residents within 0.5 miles of the facility began within 20 minutes of the accident. Many of the 939 people who went to the emergency room within 24 hours of the accident reported signs of respiratory irritation: throat burning (21.0%), shortness of breath (19.4%), sore throat (17.5%), and cough (16.4%) (Wing et al. 1991). Forced expiratory volume in 1 second (FEV_1) was <80% of predicted values in 42.3% of the 130 individuals who underwent pulmonary function testing. In another study of the Texas City residents, health effects within 1 month of the accident and 2 years after the accident were assessed in 1,994 residents who were asked to complete health questionnaires (Dayal et al. 1992). A large number of highly exposed residents reported severe symptoms of breathing problems (e.g., coughing, difficulty breathing, shortness of breath), throat problems (e.g., difficulty swallowing, burning irritation, phlegm, voice changes), and nose problems (e.g., sneezing, runny nose, problems smelling food); the prevalence of severe symptoms were 60.2, 51.9, and 40.7 for breathing, throat, and nose problems, respectively, within the first month of the accident. High prevalence of these effects were still reported 2 years after the accident; 38.5, 22.1, and 26.5 for severe breathing, throat, and nose problems, respectively. The prevalence of severe breathing, throat, and nose problems in the nonexposed population were 11.3, 6.2, and 6.4, respectively, within 1 month of the accident and 8.2, 3.3, and 4.1, respectively, 2 years after the accident. The prevalences of the breathing problems were higher in a subgroup of the high exposure group that had pre-existing respiratory problems or smoked more than two packs of cigarettes per day.

Lethality studies in animals have also reported respiratory effects in rats, mice, and guinea pigs from acute inhalation exposure to hydrogen fluoride. True respiratory effects, such as respiratory distress, pulmonary congestion, and intra-alveolar edema were generally observed at levels of at least ~50% of the LC_{50} (Haskell Laboratory 1988; Rosenholtz et al. 1963; Wohlslagel et al. 1976). These effects appear to be reversible within a week upon cessation of exposure.

A series of experiments by Dalbey et al. (1998a, 1998b) examined the acute toxicity of nonlethal concentrations of hydrogen fluoride in rats following a 2- or 10-minute exposure. In most of the experiments, a mouth-breathing model with a tracheal cannular was used to maximize delivery of hydrogen fluoride to the lower respiratory tract. A number of respiratory tract effects were found in the mouth-breathing rats, including alterations in bronchioalveolar lavage (BAL) parameters (increased total protein, myeloperoxidase, lactate dehydrogenase, β-glucuronidase, and glucose-6-phosphate dehydrogenase), impaired lung function (decreased total lung capacity, vital capacity, peak expiratory flow, forced expiratory flow at 50 and 25% of the forced vital capacity, forced expiratory volume at 0.1 second, forced vital capacity, and diffusing capacity and increased pulmonary resistance), and histological damage (necrosis and acute inflammation in trachea and acute alveolitis and perivascular/

peribronchial edema and inflammation in the lung). Rats exposed for 2 minutes manifested histological damage and BAL parameter alterations at 1,509 ppm fluoride, and impaired lung function at 4,643 ppm. No adverse respiratory effects were observed at 563 ppm fluoride. In the rats exposed for 10 minutes, histopathological alterations (necrosis of the trachea only) and BAL parameters (polymorphonuclear leukocytes and myeloperoxidase levels only) were observed at 903 ppm fluoride ; impaired respiratory function was observed at 1,676 ppm fluoride. No adverse effects were observed at 257 ppm fluoride. The respiratory effects were consistently more severe in the rats exposed for 2 minutes as compared to 10 minutes, when exposure was expressed as the product of concentration x time. In other experiments, rats were exposed for 60 minutes to hydrogen fluoride. No adverse respiratory effects were observed at 19 or 46 ppm. Respiratory effects observed in nose-breathing rats were limited to the nose. Necrosis and acute inflammation of the ventral meatus, nasal septum, and nasoturbinates were observed in rats exposed to 6,072 ppm for 2 minutes and 1,586 ppm for 10 minutes. A dramatic decrease in breathing frequency was also observed in the nose-breathing rats; within the first minute of exposure, breathing frequency was 32–35% of the preexposure levels. The decrease in breathing frequency, which is a component of reflex apnea, is a response to sensory irritation.

Similar results were observed in rats exposed to 1,235 ppm fluoride for 30 minutes. Moderate to severe fibronecrotic rhinitis and large fibrin thrombi in the submucosa and hemorrhage were observed in the nasal cavity of nose-breathing rats; no nasal lesions were observed in similarly exposed rats fitted with a tracheal cannula to simulate mouth-breathing. Epithelial, submucusal, and cartilage necrosis in the trachea, trace levels of neutrophils in the alveoli, and necrosis of the bronchi were observed in the mouth-breathing rats, but not in the nose-breathing rats, suggesting that the toxicity of hydrogen fluoride occurs at the point of entry. Reflex apnea, as evidenced by a marked decrease in breathing frequency, was observed in the nose-breathing rats. Based on differences in minute ventilation rates, the study authors estimated that the mouth-breathing rats inhaled 27% more hydrogen fluoride than the nose-breathing rats.

Pulmonary hemorrhage was noted in dogs, rabbits, and rats exposed to 31 ppm fluoride as hydrogen fluoride for 6 hours/day, 6 days/week for 5 weeks (Stokinger 1949). At 8.2 ppm fluoride, no effect was seen in rats or rabbits, and localized hemorrhages were seen in only 1/5 dogs.

Pulmonary hemorrhage, alveolar inflammation, and hyperplasia of the bronchial epithelium were observed in guinea pigs that died due to exposure to 18 ppm fluoride as hydrogen fluoride for 6–7 hours/day, 5 days/week for about 35 days (Machle and Kitzmiller 1935). This effect was not readily reversible. The one surviving guinea pig had alveolar exudates, thickening of the alveolar walls, and hemorrhages of the lungs when necropsied 9 months after the conclusion of the full 50-day exposure period. Similarly, all four rabbits exposed under the same conditions had lobular pneumonia and leucocytic infiltration of the alveolar walls, sometimes with edema and thickening of the walls, when

necropsied 7–8 months after the last exposure. No clinical signs of toxicity were reported in rabbits and weight gain was generally similar to the controls. This study is limited by the small number of animals used and the incomplete reporting of the data.

Hydrogen Fluoride and Fluoride Dusts. A study of an occupational cohort exposed to hydrogen fluoride and fluoride dusts in the pot rooms of an aluminum smelter reported a significantly lower forced expiratory volume and increased cough and sputum production in the highest exposure group, compared with controls who worked in the office or casting department and were reported to have no significant occupational exposure to air contaminants. Corrections were made for age, height, and smoking habits. The ambient air fluoride concentration in the high-exposure area was 0.2 mg fluoride/m^3 as vapor (presumably hydrogen fluoride) and 0.28 mg/m^3 "particulate fluoride." It is not clear whether the latter value represented the air concentration of fluoride in particulates or the concentration of the particulates that contain fluoride. Actual exposure was unknown because the workers wore respirators. Although urinary fluoride levels increased over the course of one work shift in the high-exposure group and not in the control group, the decrease in respiratory volume in the same time period was about the same in both groups (Chan-Yeung et al. 1983a). This effect was attributed to the fact that the exposed workers wore respirators; historical use of respirators was not reported. Because actual exposure was not known, no quantitative relationship between clinical symptoms and environmental or urinary fluoride levels could be established. There also may have been concomitant exposure to other respiratory irritants.

No studies were located regarding respiratory effects in animals following inhalation of fluoride dusts.

Cardiovascular Effects.

Hydrogen Fluoride. Cardiac arrhythmias have been seen in humans following hydrofluoric acid splashes in the face region, where both dermal and inhalation exposure were involved (Chan et al. 1987; Tepperman 1980). It is not known whether inhalation exposure alone would cause these effects. However, myocardial necrosis and congestion were observed in three rabbits following inhalation exposure of 26 ppm fluoride as anhydrous hydrogen fluoride for an unspecified period (Machle et al. 1934). The study was limited by the small sample size and undetermined exposure period.

Gastrointestinal Effects.

Hydrogen Fluoride. A population exposed to airborne hydrogen fluoride near a smelter reported nausea (22.6%) and diarrhea (21.7%). The corresponding levels reported by a control population were 6.9% and 12.1%, respectively. The total levels of gastrointestinal complaints were 70.5 and 36.2% in the subject and control populations, respectively. The subject population appears to have been derived by self-

selection and random house-to-house sampling, while the control population lived in a nonindustrial area. Although atmospheric concentrations were not presented, concentrations of fluoride in animals and plants in the area surrounding the smelter were substantially above normal. The smelter was also reported to emit metallic oxide fumes (Waldbott 1979).

Similar gastrointestinal effects (diarrhea, nausea, and vomiting) were reported by Texas residents exposed to an accidental 2-hour release of hydrogen fluoride (Dayal et al. 1992). During the first month after the accident, 38.5% of the highly exposed residents reported severe gastrointestinal effects; 15.5% of the residents still reported severe gastrointestinal effects 2 years after the accident. The occurrence of severe gastrointestinal effects among nonexposed residents was 4.5 and 2.7%, respectively, for these time periods.

Hematological Effects.

Fluorine. No studies were located on hematological effects of inhalation exposure of humans to fluorine. No effect on complete blood count parameters was observed in Osborne-Mendel rats exposed to 142 ppm for 60 minutes or 329 ppm for 15 minutes or in dogs exposed to 109 ppm for 60 minutes or 93 ppm for 15 minutes (Keplinger and Suissa 1968). These concentrations were higher than the corresponding LC_{50} values. Blood counts were monitored for 21 days postexposure. Similarly, Stokinger (1949) saw no effect on hematological parameters in dogs, rabbits, or rats following repeated exposures at concentrations up to lethal levels (31 ppm). This study did not specify which parameters were measured.

Hydrogen Fluoride. Hemograms of 20 variables (not specified) determined in the rat (30/group), rabbit (10/group), and dog (4/group) following exposure to 18 ppm fluoride for 6 hours/day, 6 days/week, for 5 weeks showed no clear changes (Stokinger 1949).

Five rabbits and two Rhesus monkeys were exposed to 18 ppm fluoride as hydrogen fluoride via inhalation 6–7 hours a day, for 50 days (Machle and Kitzmiller 1935). Blood counts were done beginning 1 week prior to exposure and ending 3 months after the final exposure. There was a small but significant decrease in erythrocyte levels in both species, but the study authors considered that the result may have been due to biological variation. Significant increases in hemoglobin levels were seen in monkeys. There was no effect on hemoglobin levels in rabbits or on leucocyte levels in either species. These experiments used only a few animals from each species, and the exposure measurement technology was not very precise.

Hydrogen Fluoride and Fluoride Dusts. No signs of hematological effects, as measured by routine blood counts, were seen in a large cohort of aluminum workers exposed to total fluoride levels below 2.5 mg/m^3

for durations of at least 10 years (Chan-Yeung et al. 1983b). Similarly, no increase in abnormal findings was seen in 74 workers exposed at a phosphate fertilizer plant (Derryberry et al. 1963). The average urinary fluoride level in the exposed group was 4.6 mg/L. Significantly reduced levels of hemoglobin were reported in Slovak children aged 6–14 years living near an aluminum smelter (Macuch et al. 1963), but no information was provided on any statistical tests used. No information was provided on air fluoride concentrations, but urinary fluoride levels were about 0.8 mg/L for 6–11-year-old children, and about 0.4 mg/L for 12–14-year-old children. In an outdated study of 78 workers exposed to cryolite, anemia was present in 11/30 subjects with pathological bone changes (Moller and Gudjonsson 1932). Blood parameters were not analyzed for the workers without bone changes.

Musculoskeletal Effects. Skeletal fluorosis is a clinical syndrome sometimes seen following chronic exposure to fluoride. It is characterized by increased x-ray bone opacity, exostoses, and calcification of ligaments. Symptoms may include bone and joint pain, and limited range of movement. Skeletal fluorosis has been reported following exposure to hydrogen fluoride, cryolite, and sodium fluoride.

Fluorine. No data were located regarding musculoskeletal effects of fluorine inhalation on humans.

Fluoride levels in the teeth of rats exposed to 18 ppm fluorine for approximately 6 hours/day, 6 days/week for 5 weeks were about 14 times the levels in controls; fluoride levels in the femur were about 6 times that of the controls (Stokinger 1949). The appearance of the teeth was characterized as corresponding to that of very mild to mild dental fluorosis. The fluoride levels in the teeth and bone at lower concentrations decreased in a concentration-related manner. Pigment changes were reported as just perceptible in animals exposed to 2 ppm fluorine.

Hydrogen Fluoride. A male exposed for 10 years to hydrogen fluoride at an alkylation unit of an oil company complained of back pains, leg pains, and loss of memory (Waldbott and Lee 1978). Initially, time away from work lessened the symptoms, but as time on the job increased, the symptoms persisted throughout periods away from work. During hospitalization following an accident, advanced osteo-arthritis of the spine was diagnosed. Based on bone fluoride measurements of 1,100 ppm 10 years after exposure ceased, the study authors concluded that the worker suffered from chronic intoxication resulting from frequent, variable exposures to airborne hydrogen fluoride. However, concomitant exposure to petroleum products, while not reported, cannot be ruled out as a cause of the pains and memory loss.

Duration- and concentration-related increases in tooth and bone fluoride levels were reported in the rat following exposure to 8.2 or 31 ppm fluoride as hydrogen fluoride for 6 hours/day, 6 days/week for

5 weeks (Stokinger 1949). The study author did not report whether there were any visible or radiological signs of dental or skeletal fluorosis.

Hydrogen Fluoride and Fluoride Dusts. Marked evidence of skeletal fluorosis was reported in workers exposed to gaseous fluoride (largely hydrogen fluoride) and fluoride dust in the pot rooms of the aluminum industry (Kaltreider et al. 1972). Individual exposure concentrations and durations were not presented. However, the estimated time-weighted average (TWA) 8-hour exposure to total fluorides for one plant ranged from 2.4 to 6.0 mg/m^3. Average urinary fluoride levels were about 9 mg/L. Exposure at a second plant was lower as a result of industrial hygiene measures; no TWA was available, but urinary fluoride levels ranged from 1.4 to 4.6 mg/L. No skeletal changes were observed at the second plant, and detailed physical examinations of the workers at both plants revealed no general health impairment. No data were presented that correlated urinary fluoride levels to the presence or absence of fluorosis.

In a follow-up study of 59 of the potroom workers at the second plant, the average preshift (after 48 hours away from work) urinary fluoride level was 2.24 mg/L (range, 1.4–3.1). The average level after 3–5 working days (postshift) was 5.68 mg/L (range, 2.7–10.4). In spite of this evidence of fluoride exposure, there was no radiological evidence of any fluoride-related bone abnormalities (Dinman et al. 1976c). Total occupational exposure ranged from 10 to 43 years. This study may provide urinary fluoride levels that are not associated with any bone effects in healthy adults. However, because only workers who remained at the high-exposure tasks for the duration of the study were examined, any sensitive population that may have found work elsewhere because of adverse health effects might have been missed.

Clinical and radiological investigations were performed for 2,258 aluminum workers exposed to fluoride for an average of 17.6 years (Czerwinski et al. 1988). The form of fluoride was not reported, but it was probably hydrogen fluoride and fluoride dust. Possible fluorosis (multiple joint pains, limited motion in at least two joints or in the spine, and initial ossifications visible on x-ray films) was found in 14% of the workers. The prevalence of definite fluorosis, with advanced limitation of movement in at least two joints or the spine, marked ossifications, and osteosclerosis, or more severe symptoms, was 6.2%. The study authors reported finding a close positive correlation between the occurrence of fluorosis and the time and level of fluoride exposure. Another health study of 2,066 workers in an aluminum smelter reported early signs of skeletal fluorosis in pot room workers employed for >10 years. No effects, however, were seen in workers exposed for <10 years. Actual airborne fluoride levels measured at the time of the health assessment were 0.2 mg/m^3 hydrogen fluoride and 0.28 mg/m^3 fluoride dusts. Historical fluoride levels were not reported, although the study authors implied that exposure levels had been below 2.5 mg/m^3 for some period (Chan-Yeung et al. 1983b).

While the above studies generally found radiologically-apparent skeletal fluorosis appearing prior to or concurrent with musculoskeletal symptoms, Carnow and Conibear (1981) found musculoskeletal symptoms in aluminum workers in the absence of radiological findings. Questionnaire answers suggested a significant increase in incidence and severity of musculoskeletal disease and fracture frequency with fluoride exposure. By contrast, there was no exposure-related increase in evidence of skeletal fluorosis on chest and spinal x-ray films. Neither radiologic data nor actual exposure levels or durations were reported. As the authors recognized, the exposure group was heterogeneous and were exposed to other chemicals, and some of the musculoskeletal symptoms may have actually been due to heavy physical labor.

Fluoride. A 58-year-old man was exposed to various fluoride compounds (chiefly sodium fluoride) for 30 years while employed in a chemical plant. The route of exposure was presumed to be inhalation with some concomitant dermal exposure. He had fluoride deposits in almost all of his bones, but the main accumulation was in the vertebrae, ribs, and pelvic bones (McGarvey and Ernstene 1947). No other effects were observed that could be attributed to the fluoride exposure. Another man exposed almost daily for 18 years to a finely ground rock phosphate dust containing 3.88% fluoride had "thicker and heavier" bones with a white, chalky material covering the surface as observed by x-ray (Wolff and Kerr 1938). His bones also appeared to be more opaque when compared by x-ray to normal bones.

No studies were located regarding musculoskeletal effects in animals after inhalation exposure to fluoride.

Hepatic Effects.

Fluorine. No studies were located regarding hepatic effects of fluorine inhalation in humans. Mice exposed to fluorine exhibited coagulation necrosis of the liver, periportal hemorrhages, and diffuse cloudy swelling (Keplinger and Suissa 1968). These effects were generally observed after exposure to concentrations of 195, 144, 116, or 80 ppm fluoride for 5, 15, 30, or 60 minutes, respectively. Damage became apparent 7–14 days after exposure. Liver congestion was reported in dogs, but not in other species subjected to repeated exposures to a lethal concentration of fluorine (18 ppm 6 hours/day, 6 days/week for 5 weeks) (Stokinger 1949).

Hydrogen Fluoride. Ten animals (five rabbits, three guinea pigs, and two Rhesus monkeys) were exposed via inhalation to 18 ppm fluoride as hydrogen fluoride 6–7 hours/day for 50 days (Machle and Kitzmiller 1935). Fatty degeneration of the liver parenchyma, scattered focal necroses, and fibroblastic encroachment of periportal spaces were observed in the guinea pigs. Two of the three guinea pigs began losing weight after about 145 hours of exposure, were withdrawn from the exposure regimen, and died about 2 weeks later. Generalized fatty changes were also seen in two of four rabbits sacrificed 7 months

after exposure termination. These experiments used only a few animals from each species, and the exposure measurement technology was not very precise.

Hydrogen Fluoride and Fluoride Dusts. The occupational health study by Chan-Yeung et al. (1983b) discussed above revealed no adverse effects on liver function, as measured by levels of total bilirubin, serum glutamic oxaloacetic transaminase (SGOT), and alkaline phosphatase.

Renal Effects.

Fluorine. No studies were located regarding renal effects of fluorine inhalation in humans. Mice exposed to fluorine exhibited focal areas of coagulation necrosis in the renal cortex and focal areas of lymphocyte infiltration in the cortex and medulla following exposure to 114 ppm for 5 minutes, 82 ppm for 15 or 30 minutes, or 55 ppm for 60 minutes (Keplinger and Suissa 1968). Damage became apparent 7–14 days postexposure.

Hydrogen Fluoride. Pathologically elevated serum creatinine and urea levels were seen 24 hours after accidental dermal and inhalation exposure to a mixture of 70–80% sulfuric acid and 10% hydrofluoric acid at 150 EC (Braun et al. 1984). Neither the effect of the sulfuric acid nor the exposure levels were known.

Degeneration and necrosis of the renal cortex was reported in 27/30 rats exposed to 31 ppm fluoride as hydrogen fluoride for 6 hours/day, 6 days/week for 5 weeks, but not in rats exposed to 8.2 ppm fluoride (Stokinger 1949). Pathological examination of rabbits and guinea pigs (n=3/species/exposure level) exposed to hydrogen fluoride revealed tubular necrosis, congestion, and edema (Machle et al. 1934). A variety of different exposure levels and durations were tested, but the levels at which exposure-related effects were seen were not reported. Rabbits (n=4) exposed via inhalation to 18 ppm fluoride as hydrogen fluoride 6–7 hours/day for 50 days, developed degeneration and necrosis of convoluted tubules, accompanied by fibrous tissue replacement of cortical tissues (Machle and Kitzmiller 1935). Degenerative and inflammatory changes were also seen in the single exposed monkey at necropsy. The experiments described in both of these papers used a small number of animals, and no control data were presented.

Hydrogen Fluoride and Fluoride Dusts. Increased incidence of albuminuria ($p<0.1$) was observed in phosphate fertilizer plant workers with an average urinary fluoride level of 4.6 mg/L (Derryberry et al. 1963). However, the testing method used in this study is considered to be hypersensitive (Dinman et al. 1976a), and several other studies have found no effects. No signs of renal effects, as measured by standard renal function tests, were seen in a large cohort of aluminum workers exposed to total fluoride

levels estimated to be below 2.5 mg/m^3 (Chan-Yeung et al. 1983b). Two other studies of aluminum workers failed to find an increase in the incidence of albuminuria (Dinman et al. 1976c; Kaltreider et al. 1972). Average postshift urinary fluoride levels were #5.68 mg/L (Dinman et al. 1976c) and #9.6 mg/L (Kaltreider et al. 1972). The exposed population included workers exposed to estimated air fluoride levels of 4–6 mg/m^3 (time-weighted average), of which 50% was gaseous fluoride (presumably hydrogen fluoride) (Kaltreider et al. 1972).

The weight-of-evidence indicates that typical inhalation occupational exposure to hydrogen fluoride and fluoride dust is not nephrotoxic. The overall animal data indicate that inhalation exposure to sufficiently high levels of hydrogen fluoride or fluorine can cause kidney damage, but the relevance to human health and the potential nephrotoxic level cannot be determined because of generally incomplete human and animal data. In addition, only one animal experiment was located that conducted a histopathic exam following fluorine exposure.

Endocrine Effects. No studies were located regarding endocrine effects in humans or animals after inhalation exposure to fluorine, hydrogen fluoride, or fluoride.

Dermal Effects. Dermal effects (irritation of the skin) have been observed in humans following exposure to airborne fluoride. Because the effects are believed to be due to irritation caused by direct dermal contact with these gases, they are discussed under Dermal Exposure (see Section 3.2.3).

Ocular Effects. As with dermal effects, ocular effects such as lacrimation and reddened conjunctiva have been reported in humans and animals exposed to airborne fluorine and hydrogen fluoride. These effects are discussed under Dermal Exposure (see Section 3.2.3) because they are due to direct contact rather than from absorbed fluoride.

Body Weight Effects.

Fluorine. Decreased weight gain was observed in rats, guinea pigs, and rabbits exposed to 18 ppm fluorine for about 6 hours/day, 6 days/week for 5 weeks (Stokinger 1949). While a decreased weight gain in the high-exposure group compared to the low-exposure groups is clear, no control animals were used and the lowest exposure level that would result in a significant change was not established.

Hydrogen Fluoride. Pronounced weight loss shortly before death was observed in rats exposed to a lethal level of hydrogen fluoride (31 ppm fluoride for 6 hours/day, 6 days/week for 5 weeks). Guinea pigs exposed under the same conditions lost weight following the third exposure week, even though there were no deaths (Stokinger 1949). While a decrease compared to the low-exposure level group is clear, no

control animals were used and the lowest exposure level that would result in a significant change was not established. Animals surviving a lethal exposure exhibited a body weight loss of 10–15% for up to a week after exposure (Rosenholtz et al. 1963; Stavert et al. 1991).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to fluorine, hydrogen fluoride, or fluoride.

3.2.1.4 Neurological Effects

Fluorine. No studies were located regarding neurological effects in humans of fluorine following inhalation exposure. Dogs exposed to 5 or 18 ppm for 6 hours/day, 6 days/week for up to 35 days had seizures prior to death (Stokinger 1949). Because no further details were available, the neurotoxic potential of fluorine cannot be evaluated.

Hydrogen Fluoride. The threshold of the light adaptive reflex was measured as a marker for neurological effects in three subjects following exposure to hydrogen fluoride at concentrations of 0.02, 0.03, or 0.06 ppm fluoride (Sadilova et al. 1965). While the threshold level was determined to be 0.03 ppm, it is not clear whether this response is due to irritation of mucous membranes or is the result of an effect on cerebral cortical function. Details of atmosphere generation were not provided.

Exposure to concentrations at about 50% of the LC_{50} values was reported to cause general weakness and decreased activity in rats of a Wistar-derived (Rosenholtz et al. 1963). Albino rats given 24-hour exposures to either 0.03 or 0.1 ppm fluoride as hydrogen fluoride for 5 months developed central nervous system dysfunctions, as evidenced by diminished conditioned responses and increased time before motor nerve response. Histological studies showed changes in the nerve cell synapses of only those animals exposed to 0.1 ppm. A concentration of 0.01 ppm was found to be without effect on conditioned responses, latency in motor nerve response, or neurohistological parameters. When additional stresses were added (alcohol, 24-hour starvation), the conditioned responses were extinguished more frequently (Sadilova et al. 1965). Some recovery in conditioned responses was seen following a 1-month recovery period in the animals exposed to 0.1 ppm. Animals exposed to 0.03 ppm recovered completely.

All reliable LOAEL values for neurological effects of exposure to hydrogen fluoride in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.1.5 Reproductive Effects

Fluorine. Rats exposed to 18 ppm fluorine, 6 hours/day, 6 days/week for 5 weeks showed testicular degeneration (Stokinger 1949). No further details were available. It is not clear whether this effect was seen both in animals that died and in those that survived.

Hydrogen Fluoride. All four dogs exposed to 18 ppm fluoride as hydrogen fluoride for 6 hours/day, 6 days/week for 5 weeks developed degenerative testicular changes and ulceration of the scrotum (Stokinger 1949). This effect was not seen at 8.2 ppm, or in rabbits or rats at either exposure level. No further details were available. Furthermore, it is not clear whether this is a systemic effect or a result of irritation from dermal contact with the gas.

No studies were located regarding reproductive effects in humans after inhalation exposure to fluorine, hydrogen fluoride, or fluoride, and no studies were located regarding reproductive effects in animals after inhalation exposure to fluoride.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to fluorine, hydrogen fluoride, or fluoride.

3.2.1.7 Cancer

Hydrogen Fluoride and Fluoride Dusts. Most occupational exposure to fluoride occurs as a result of inhalation of hydrofluoric acid fumes or dust from cryolite or fluorspar. A cohort of cryolite workers in Denmark was reported to have an increase in mortality and morbidity from respiratory cancer compared with the national average (Standardized Mortality Ratio of 2.52 [95% confidence limit between 1.40 and 4.12], Standardized Incidence Ratio of 2.5 [95% confidence limit between 1.6 and 3.5]) (Grandjean et al. 1985). The study authors stated that the increase can be explained by the fact that the respiratory cancer death rate for the Copenhagen area is about twice the national average for the birth cohorts from 1890 to 1929, so that comparison with national rates may not be appropriate. Respiratory cancer rates for the workers were slightly higher than those of the general population of Copenhagen, but the difference was not significant. No explanation for the high Copenhagen rates was offered.

Increased lung cancer rates have been reported in several studies of aluminum industry workers (Andersen et al. 1982; Gibbs and Horowitz 1979; Milham 1979), but no correction was made for smoking or concurrent exposure to tars and polycyclic aromatic hydrocarbons. Similarly, fluorspar miners had

increased lung cancer rates, but they were also exposed to elevated radon levels (deVilliers and Windish 1964). A cohort study of 21,829 workers in aluminum reduction plants for $5 years did not find an increase in lung cancer, but did report an increase in mortality due to pancreatic cancer, lymphohematopoietic cancers, genitourinary cancer, and nonmalignant respiratory disease (Rockette and Arena 1983). Only the effect on pancreatic cancer rates was statistically significant. Increases in incidence of hematopoietic cancers and respiratory disease were also reported by Milham (1979). Because of the confounding factors mentioned above, and because no breakdown was done by fluoride exposure, these studies are of questionable relevance to the issue of possible carcinogenicity of inhalation exposure to hydrogen fluoride and/or fluorides.

A study was published describing a positive relationship between increased lung cancer occurrence and exposure to fluoride among individuals residing near, or working in, the steel industry (Cecilioni 1972). Possible occupational exposures to other carcinogenic substances from steel and other industries were not considered. Carcinogenicity via inhalation of fluoride is not considered to be likely by most investigators reporting in the existing literature.

No studies were located regarding cancer in animals after inhalation exposure to fluorine, hydrogen fluoride, or fluoride.

3.2.2 Oral Exposure

Because fluorine and hydrogen fluoride are gases, oral exposure to these substances occurs only concomitant with inhalation exposure. Oral exposure to hydrofluoric acid has been reported very rarely. Except where otherwise indicated, the following sections on oral exposure refer to oral exposure to fluoride.

Much of the research on fluoride exposure in humans has focused on the ingestion of fluoride through supplemented public drinking water supplies. Additional information comes from studies of areas with high natural fluoride levels. Drinking water levels of other minerals may differ between artificially fluoridated areas and areas with naturally high fluoride levels. Chronic fluoride ingestion can affect bone structure. High skeletal fluoride levels can lead to skeletal fluorosis, a disease characterized by increased bone density. The most severe form of fluorosis can result in crippling deformities, but this is extremely rare in the United States. Teeth mottling is another chronic effect that can occur in children exposed to fluoride during tooth formation. A review of studies concerning dental fluorosis found that in the surveyed cities with water containing 0.7–1.2 ppm (the level to which water is fluoridated), an average of 10–20% of the children had barely noticeable changes in their teeth, while up to 1% had brown spots due

to fluoride (DHHS 1991). Based on this result, the study concluded that total fluoride intake from multiple sources has risen since the optimal water fluoridation levels were set.

Much of the data regarding toxic effects of oral exposure to fluoride were obtained from studies using sodium fluoride. Fluoride is often added to water in the form of hydrofluosilicic acid, so exposure to this chemical is included in some epidemiological studies. Other studies investigate oral exposure to calcium fluoride, hydrofluoric acid, cryolite, and fluoride in rock phosphate. For all forms of fluoride discussed, doses are reported as amount of the fluoride ion.

Conflicting results have been obtained from experiments addressing whether fluorine is an essential element. Much of this conflict appears to result from the great difficulty in preparing an animal diet that has negligible amounts of fluoride, but otherwise allows normal animal growth and development. Different conclusions have been reached by different official organizations. The Institute of Medicine has derived adequate intake values ranging from 0.01 to 4 mg/day to reduce the occurrence of dental caries. Adequate intake values broken down by age group are 0–6 months, 0.01 mg/day; 7–12 months, 0.5 mg/day; 1–3 years, 0.7 mg/day; 4–8 years, 1 mg/day; 9–13, 2 mg/day; 14–18 years, 3 mg/day; 19 years and older, 4 mg/day (males) and 3 mg/day (females); pregnancy, 3 mg/day; and lactation, 3 mg/day (IOM 1997). The World Health Organization (WHO) also lists fluorine as essential for animal life, without providing supporting data (WHO 1973).

As discussed in Section 3.2.2.6, there have been suggestions that fluoride can aid fertility by improving intestinal absorption of iron and other trace elements (Messer et al. 1973; Tao and Suttie 1976). In a study where fluoride was rigorously removed from dietary components, a total of 110 Wistar rats were observed over the course of four generations (Maurer and Day 1957). There were no adverse effects compared to controls that received the same diet and 0.28 mg fluoride/kg/day in drinking water. Animals fed the low-fluoride diet were healthy, had sleek coats and healthy teeth, and had similar weight gains to those of the controls. Low success in bringing pups to weaning (50%) was reported for both the low-fluoride and control groups. No fluoride was detectable in the diet (detection limit not reported), and fluoride levels in femurs were #8.8 ppm fluoride in bone ash. In a more recent study, dose-dependent increases in daily weight gain of F344 rats were observed when a low-fluoride diet was supplemented with fluoride (Schwarz and Milne 1972). The fluoride provided by the basal diet varied, but was sometimes 0.023 mg/kg/day and occasionally dropped below 0.002 mg/kg/day. However, the results are likely to be due to other nutritional deficiencies that were partially compensated by fluoride. Rats in both the control and low-fluoride groups had shaggy fur, loss of hair, and seborrhea. Fluoride was only partially effective in correcting the bleached incisors found in the low-fluoride group. Bleached incisors have been related to deficiencies of calcium, phosphorus, magnesium, iron, and vitamins E, D, and A. None of these studies provide strong evidence that fluoride is an essential element.

3.2.2.1 Death

Fluoride. Based on numerous incidents of fatal fluoride poisoning, Hodge and Smith (1965) estimated the certainly lethal dose (CLD) (without treatment) for a 70-kg man at 5–10 g sodium fluoride, or 32–64 mg fluoride/kg body weight. The faster uptake of fluoride to the bone in children helps to clear fluoride from the bloodstream, so Heifetz and Horowitz (1986) did not believe that the CLD for children would be lower than that for adults. The safely tolerated dose has been reported as 8–16 mg fluoride/kg, but no support was provided for this conclusion (Heifetz and Horowitz 1986). As indicated below, this dose may not be tolerated by very small children.

Fatal ingestion of sodium fluoride has been reported as early as 1899 (Sharkey and Simpson 1933). A summary of early fatalities indicates that the primary symptoms were the sudden onset of nausea and vomiting, accompanied by burning, cramp-like abdominal pains and diarrhea. Clonic convulsions and pulmonary edema were reported in some cases; the pulmonary edema may have been due to aspiration of vomitus. While a few of these deaths were suicides, most of them resulted from accidental exposure to sodium fluoride when containers of insecticide were mistaken for baking powder or epsom salts.

More recent information includes the case report of a 3-year-old boy who swallowed 200 sodium fluoride tablets (1 mg fluoride each) for a dose of 16 mg fluoride/kg body weight (Eichler et al. 1982). Immediately after ingestion, he vomited and appeared to recover, but he collapsed 4 hours later. The boy died 7 hours after fluoride ingestion. Upon autopsy, hemorrhagic edema of the lungs, hemorrhagic gastritis, and massive cerebral edema were observed. The hemorrhagic edema observed in the lungs was probably due to aspiration of the gastric contents. Cloudy swelling was observed in the cells of the liver, heart, and kidney. In another case, a 27-month-old child died 5 days after ingesting about 100 fluoride tablets, for a dose of about 8 mg fluoride/kg body weight (Whitford 1990). Based on this case and weight tables for 3-year-old boys, Whitford (1990) calculated a probable toxic dose of about 5 mg fluoride/kg body weight.

A comparison of death rates between U.S. cities with fluoridated water and those with nonfluoridated water found no association between fluoride and increased death rate (Erickson 1978). It is difficult to draw definitive conclusions from this study because it is limited by dissimilarities between the populations, which led to a need for multiple adjustments.

In rats, LD_{50} values for sodium fluoride administered by oral gavage range from 31 to 101 mg fluoride/kg (DeLopez et al. 1976; Lim et al. 1978; Skare et al. 1986). These LD_{50} values for rats are for different strains with variations in weight. Gender differences may also account for the reported differences in LD_{50} values; an LD_{50} of 101 mg fluoride/kg was reported for male Sprague-Dawley rats weighing

150–290 g, while LD_{50} values for female Sprague-Dawley rats weighing 112–184 and 200–359 g were 52 and 31 mg/kg, respectively. An LD_{50} of 44.3 mg fluoride/kg was reported for mice (Lim et al. 1978).

Hydrofluoric Acid. Six deaths were reported to have occurred between 1 and 6 hours following accidental or intentional ingestion of a rust remover containing hydrofluoric acid (Menchel and Dunn 1984). No dose levels of fluoride were reported. At autopsy, severe hemorrhagic gastritis was noted in all cases. In one case, hemorrhage and necrosis of the pancreas were also noted. A fatal case of hydrofluoric acid ingestion occurred when a 29-year-old man drank a mouthful, thinking it was water (Manoguerra and Neuman 1986). In spite of immediate vomiting, respirations were shallow within an hour, and the patient died within 2 hours of exposure. Serum calcium and SGOT levels were markedly depressed. Serum fluoride level was 35 ppm. Another study reported six deaths due to hydrofluoric acid ingestion (Menchel and Dunn 1984). The major symptoms reported were nausea, thirst, and ulcerations of the buccal mucosa, followed by the rapid onset of tetany and coma.

All reliable LD_{50} and LOAEL values for death in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-3.

3.2.2.2 Systemic Effects

The predominant systemic effects that have been observed following acute oral exposures to sodium fluoride are hypocalcemia (resulting in tetany and ventricular fibrillation, among other effects), hyperkalemia, and gastrointestinal pain; fluorosis is the major effect of chronic exposure.

No studies were located regarding dermal or ocular effects in humans or animals after oral exposure to fluorine, hydrogen fluoride or fluoride.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-3.

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to fluorine, hydrogen fluoride, or fluoride.

Congestion, the presence of edema fluid, and desquamation of respiratory epithelium were observed in the lungs of rabbits exposed to 4.5 or 9 mg fluoride/kg/day as sodium fluoride in the diet for 6 months (Purohit et al. 1999). Inflammatory cell infiltrates, congestion, and desquamated epithelium were also observed in the large bronchi and trachea of rabbits fed 9 mg fluoride/kg/day. Necrosis of the lung parenchyma was also observed in two high-dose rabbits that died before the end of the study.

Table 3-3. Levels of Significant Exposure to Fluoride - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Human	1d 1x/d (C)				16 (1 child)	Eichler et al. 1982 sodium fluoride
2	Rat (Sprague-Dawley)	1 d 1x/d (GW)				54 (LD_{50} for 80 g rats)	DeLopez et al. 1976 sodium fluoride
						52 (LD_{50} for 150 g rats)	
						31[b] (LD_{50} for 250 g rats)	
3	Rat (Rochester)	1 d 1x/d (GW)				51.6 (LD_{50})	Lim et al. 1978 sodium fluoride
4	Rat (Sprague-Dawley)	1 d 1x/d (GW)				101.3 (LD_{50})	Skare et al. 1986 sodium fluoride
5	Mouse (Swiss)	1 d 1x/d				44.3 (LD_{50})	Lim et al. 1978 sodium fluoride
	Systemic						
6	Rat	2wk (W)	Musc/skel		9.5 (decreased modulus of elasticity)		Guggenheim et al. 1976 sodium fluoride

Table 3-3. Levels of Significant Exposure to Fluoride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Reproductive						
7	Mouse	5d 1x/d (G)		32			Li et al. 1987a sodium fluoride
	Developmental						
8	Rat (Sprague-Dawley)	Gd 6-15 daily (W)		12.26			Heindel et al. 1996 sodium fluoride
9	Rabbit (New Zealand)	Gd 6-19 daily (W)		13.21			Heindel et al. 1996 sodium fluoride

Table 3-3. Levels of Significant Exposure to Fluoride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Death						
10	Mouse (B6C3F1)	6 mo daily (W)				67 (increased mortality)	NTP 1990 sodium fluoride
11	Mouse	6mo ad lib (W)				300[c] M (mortality) 600 F (mortality)	NTP 1990 sodium fluoride
	Systemic						
12	Rat	2 mo 7d/wk 24hr/d (W)	Endocr		0.5 (decreased thyroxine levels; increased T3-resin uptake ratio)		Bobek et al. 1976 sodium fluoride
13	Rat (Sprague-Dawley)	7d/wk 24hr/d (W)	Musc/skel		10.5 (decr mineral content and incr proline in tooth enamel matrix)		DenBesten and Crenshaw 1984 sodium fluoride
14	Rat (Wistar)	5 wk (W)	Musc/skel	13	19 (histological fluorosis; decr bone growth)		Harrison et al. 1984 sodium fluoride
15	Rat (Fischer- 344)	6 mo daily (W)	Gastro		7 (hyperplasia of glandular stomach)		NTP 1990 sodium fluoride
			Hepatic	20			
			Renal	20			

Table 3-3. Levels of Significant Exposure to Fluoride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
16	Rat	30d (W)	Musc/skel		14 (delayed healing of broken bones)		Uslu 1983 sodium fluoride
17	Mouse	280 d daily (W)	Hepatic		0.95 (pale, granular hepatocytes with fatty vacuoles)		Greenberg 1982a sodium fluoride
18	Mouse	280d (W)	Renal		1.9 (nephron degeneration)		Greenberg 1986 sodium fluoride
19	Mouse	4 wk 7d/wk daily (W)	Musc/skel		0.80 (incr bone formation rate; slight decr bone calcium)		Marie and Hott 1986 sodium fluoride
20	Mouse (B6C3F1)	6 mo daily (W)	Cardio			67 (multifocal mineralization and degeneration of the myocardium)	NTP 1990 sodium fluoride
			Musc/skel		5.6 M (increased osteoid in femur and tibia)		
			Hepatic		67 (megaolocytosis and syncytial alteration)		
			Renal			67 (multifocal nephrosis)	
			Bd Wt		67 (20% decr bw gain)		
21	Mouse	35 d 1x/d (GW)	Hemato		5.2 (decr RBC and hemoglobin, incr WBC)		Pillai et al. 1988 sodium fluoride
			Bd Wt		5.2 (decr body weight)		
22	Rabbit (NS)	6 mo daily (F)	Resp		4.5 (congestion, edema fluid, desquamation of respiratory epithelium in lungs)		Purohit et al. 1999 sodium fluoride

Table 3-3. Levels of Significant Exposure to Fluoride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Neurological							
23	Rat (Sprague-Dawley)	6 wk daily (W)			6 F (altered spontaneous behavior)		Mullenix et al. 1995 sodium fluoride
24	Rat (Sprague-Dawley)	6 wk daily (W)		5.5 F	7.5 F (altered spontaneous behavior)		Mullenix et al. 1995 sodium fluoride
25	Rat (Wistar)	60 d daily (GW)			9 (decr spontaneous activity)		Paul et al. 1998 sodium fluoride
Reproductive							
26	Rat (CD)	60 d 7d/wk (F)			2.3 (decr seminiferous tubule diameter)	4.5 (50% reduction in fertility, decr in percentage of seminiferous tubules containing spermatozoa and decr testosterone levels)	Araibi et al. 1989 sodium fluoride
27	Rat (NS)	daily 30 d (GW)				2.3 (decreased fertility and sperm counts)	Chinoy et al. 1992 sodium fluoride
28	Rat (Charles Foster)	30 or 50 days d (F)				4.5 (decreased sperm motility and count)	Chinoy et al. 1995 sodium fluoride
29	Rat (Wistar)	daily 6 wk (W)		21			Krasowska and Wlostowski 1992 sodium fluoride

Table 3-3. Levels of Significant Exposure to Fluoride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
30	Rat (Wistar)	daily 16 wk (W)			7.5 (seminiferous tubule atrophy)		Krasowska and Wlostowski 1992 sodium fluoride
31	Rat	3 mo 7d/wk (F)		23			Marks et al. 1984 sodium fluoride
32	Rat Charles Foster	daily 50 d (GW)			4.5 (decr testosterone levels and Leydig cell diameter)		Narayana and Chinoy 1994 sodium fluoride
33	Rat (Sprague-Dawley)	daily (W)		16			Sprando et al. 1997 sodium fluoride
34	Rat (Sprague-Dawley)	daily (W)		16			Sprando et al. 1998 sodium fluoride
35	Mouse (Swiss)	30 d daily (F)				4.5 (decr sperm motility and count and infertility)	Chinoy and Sequeira 1992 sodium fluoride
36	Mouse (Swiss-Webster)	25 wks (W)		9.5		19 (nearly complete infertility)	Messer et al. 1973 sodium fluoride
37	Mouse	35 d 1x/d (GW)		5.2			Pillai et al. 1988 sodium fluoride

Table 3-3. Levels of Significant Exposure to Fluoride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
38	Gn Pig (NS)	30 d daily (GW)				4.5 (decr sperm motility and viability)	Chinoy et al. 1997 sodium fluoride
	Developmental						
39	Rat (CD)	Gd 1-20 daily (W)		11.2	11.4 (incr in average number of fetuses per litter with 3+ skeletal variations)		Collins et al. 1995 sodium fluoride
40	Rat (Sprague-Dawley)	28 wk 7d/wk 24hr/d (W)		21			Ream et al. 1983 sodium fluoride

Table 3-3. Levels of Significant Exposure to Fluoride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Systemic						
41	Human	4 yr (C)	Musc/skel		0.56[d] (increased fracture rate)		Riggs et al. 1990 sodium fluoride
42	Rat (Fischer- 344)	103 wk (W)	Resp	3.9			NTP 1990 sodium fluoride
			Cardio	3.9			
			Gastro	3.9			
			Hemato	3.9			
			Musc/skel	2.5	4.3 (osteoscleosis)		
			Hepatic	3.9			
			Renal	3.9			
			Bd Wt	3.9			
43	Mouse (B6C3F1)	103 wk (W)	Resp	7.6			NTP 1990 sodium fluoride
			Cardio	7.6			
			Gastro	7.6			
			Hemato	7.6			
			Musc/skel	4.3 M	7.6 M (dentine dysplasia)		
			Hepatic	7.6			
			Renal	7.6			
			Bd Wt	7.6			
44	Rabbit	24 mo 1x/d (GW)	Gastro		5 (roughened duodena mucosa)		Susheela and Das 1988 sodium fluoride

Table 3-3. Levels of Significant Exposure to Fluoride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
45	Rabbit	7-12 mo 1x/d (G)	Hemato		4.52 (decr leukocyte and hemoglobin levels)		Susheela and Jain 1983 sodium fluoride
46	Mink	382 d 24hr/d (F)	Musc/skel		5 (mottled and brittle kit teeth)	9.1 (sagittal crests deformed, 3/6 adults)	Aulerich et al. 1987 sodium fluoride
	Immunological/Lymphoreticular						
47	Rabbit (albino)	18 mo 1x/d (G)			4.5 (decr primary and secondary antibody titers)		Jain and Susheela 1987 sodium fluoride
	Reproductive						
48	Mouse	3 gen (F)		13			Tao and Suttie 1976 sodium fluoride
49	Rabbit (NS)	daily 18 mo (GW)				4.5 M (structural damage of the spermatid and epididymal spermatozoa)	Kumar and Susheela 1994 sodium fluoride
50	Rabbit (NS)	daily 20 or 23 mo (GW)				4.5 M (structural damage of the spermatid and epididymal spermatozoa)	Kumar and Susheela 1995 sodium fluoride
51	Rabbit (NS)	daily 18 or 29 mo (GW)				4.5 (complete cessation of spermatogenesis)	Susheela and Kumar 1991 sodium fluoride
52	Rabbit (New Zealand)	daily 18 or 23 mo (GW)			4.5 (Leydig cell damage)		Susheela and Kumar 1997 sodium fluoride

Table 3-3. Levels of Significant Exposure to Fluoride - Oral (continued)

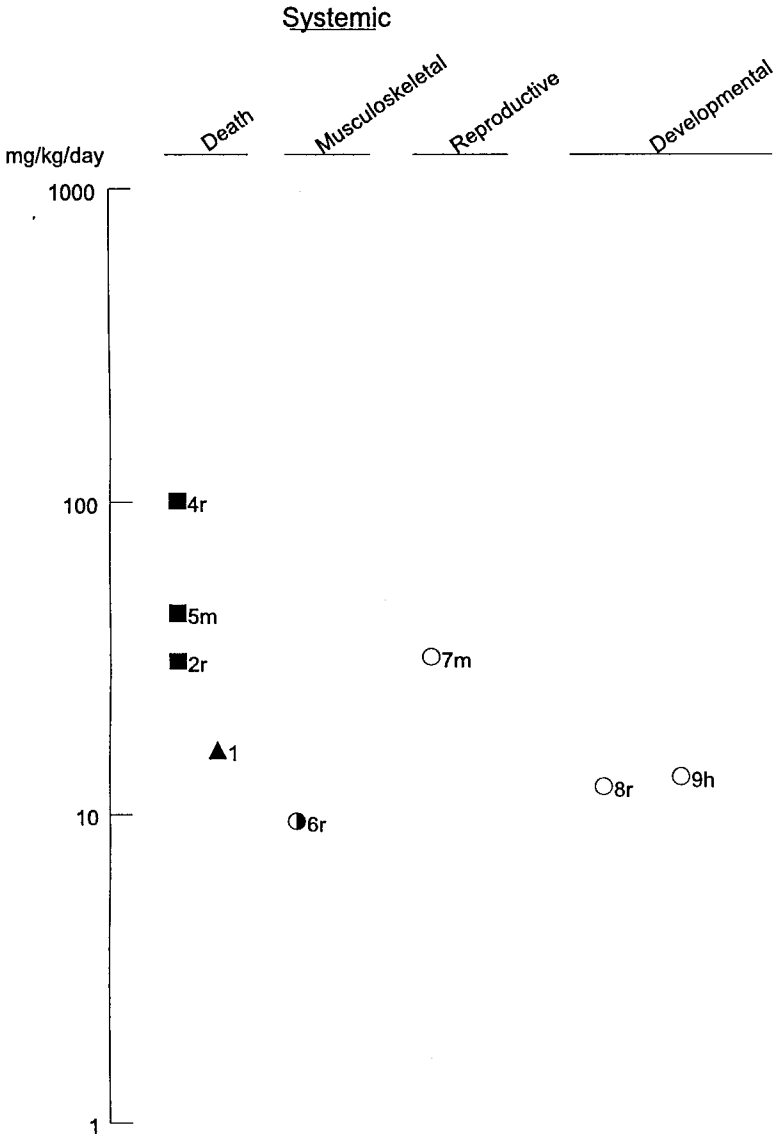
Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
53	Mink	382 d daily (F)		9.1			Aulerich et al. 1987 sodium fluoride
	Cancer						
54	Rat (Fischer- 344)	103 wk (W)				2.4 M (osteosarcoma of bone)	NTP 1990 sodium fluoride

[a]The number corresponds to entries in Figure 3-3.
[b]Only this dose level, the most sensitive dose level, is plotted in Figure 3-3.
[c]Differences in levels of health effects and cancer effects between males and females are not indicated in Figure 3-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[d]Used to derive a chronic oral minimal risk level (MRL) of 0.06 mg/kg/day; dose divided by an uncertainty factor of 10 for use of a LOAEL identified in a sensitive subpopulation.

ad lib = ad libitum; Bd Wt = body weight; (C) = capsule); d = day(s); decr = decrease; Endocr = endocrine; (F) = feed; F = female(s); (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; gen = generation(s); (GW) = gavage in water; Hemato = hematological; hr = hour(s); incr = increase; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = males; mg/kg/day = milligram per kilogram per day; mo = month(s); Musc/skel = muscular/skeletal; NOAEL = no-observed-adverse-effect level; RBC = red blood cell(s); Resp = respiratory; T3 = triiodothyronine; (W) = water; WBC = white blood cell(s); wk = week(s); x = time

Figure 3-3. Levels of Significant Exposure to Fluoride - Oral

Acute (≤14 days)



c-Cat, d-Dog, r-Rat, p-Pig, q-Cow; -Humans, k-Monkey, m-Mouse, h-Rabbit, a-Sheep; f-Ferret, j-Pigeon, e-Gerbil, s-Hamster, g-Guinea Pig; n-Mink, o-Other

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-3. Levels of Significant Exposure to Fluoride - Oral (*continued*)

Intermediate (15-364 days)

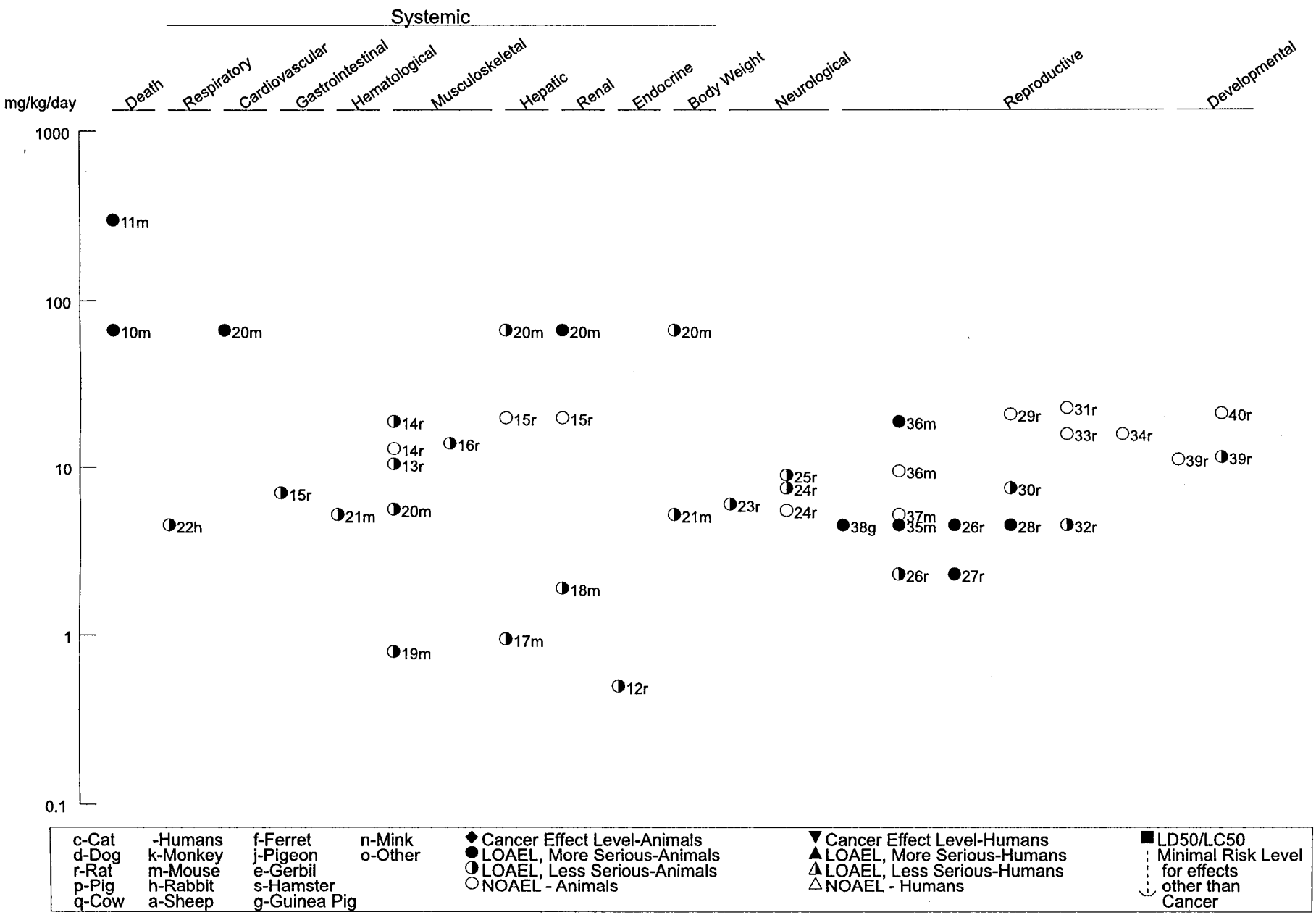
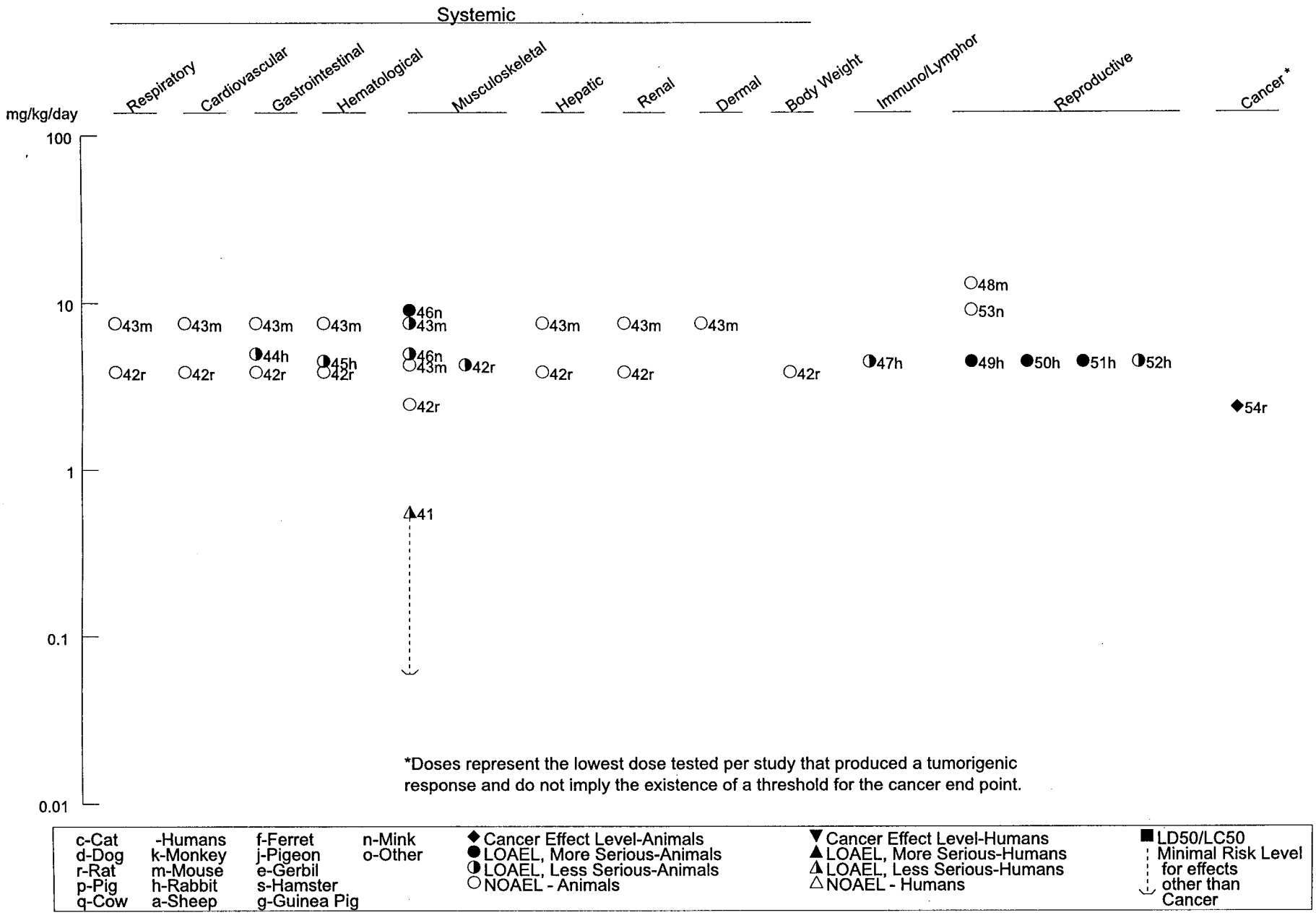


Figure 3-3. Levels of Significant Exposure to Fluoride - Oral (*continued*)

Chronic (≥365 days)



Cardiovascular Effects. The cardiovascular effects of fluoride have been attributed to hypocalcemia and hyperkalemia caused by high fluoride levels. Fluoride can bind with serum calcium if the dose is sufficient and cause hypocalcemia. Calcium is necessary for the functional integrity of the voluntary and autonomic nervous systems. Hypocalcemia can cause tetany, decreased myocardial contractility, and possibly cardiovascular collapse (Bayless and Tinanoff 1985). Hyperkalemia has been suggested as the cause of the repeated episodes of ventricular fibrillation and eventual death that are often encountered in cases of fluoride poisoning (Baltazar et al. 1980).

Approximately 2 hours after ingestion of 120 g of roach powder (97% sodium fluoride) in an unsuccessful suicide attempt, a 25-year-old male had severe toxic reactions that included tetany, multiple episodes of ventricular fibrillation, and esophageal stricture (Abukurah et al. 1972). Within 14 hours following exposure, the patient experienced 63 episodes of ventricular fibrillation.

In two epidemiological studies, fluoride in the drinking water did not increase the mortality rates from cardiovascular effects. One of these studies was a report of 428,960 people in 18 areas of "high" natural fluoride (0.4–>3.5 ppm) in England and Wales and 368,580 people in control areas (<0.2 ppm fluoride). The water supply for 52% of the "high" fluoride population had average fluoride levels of $1 ppm (Heasman and Martin 1962). Results indicated that there were no significant differences between areas with different fluoride levels in mortality due to coronary disease, angina, and other heart disease, as evidenced by standard mortality ratios (SMRs). The second study (Hagan et al. 1954) examined 32 pairs of cities in the United States that contained 892,625 people in the high fluoride areas and 1,297,500 people in the control cities. A positive relationship between heart disease and water fluoridation was reported, but these authors did not adjust for a doubling of the members of this population over 75 years old during the period of fluoridation under study (Jansen and Thomson 1974). In addition, this study lacked statistical analysis and drew conclusions regarding trends that were not obvious from the data presented. The large variation in the presented data was not discussed. Doses of fluoride are difficult to estimate for large populations, however, because most people are potentially exposed to fluoride through a variety of sources, such as food, beverages, medicine, and dental products.

By contrast, a comparison of Bartlett and Cameron, two Texas towns with water supplies containing 8 and 0.4 ppm fluoride, respectively, found a significantly higher rate of cardiovascular system abnormalities in the town with the lower fluoride level (Leone et al. 1954). The authors attributed the finding of a significant result to the number of statistical tests that were conducted in the study. However, it is interesting to note that a study of 300 North Dakota residents who drank water containing 4–5.8 ppm and 715 people who drank water containing 0.15–0.3 ppm found a lower incidence of calcification of the aorta in the high-fluoride group (Bernstein et al. 1966). Significant differences were found in 45–54-year-old males ($p<0.05$), as well as in males aged 55–64 and 65+ years ($p<0.01$). This effect was

not due solely to differences in age distribution, because the incidence in the 55–64-year-old, high-fluoride group was lower than the incidence in the 45–54-year-old, low-fluoride group. A crude analysis also found no association with milk and cheese consumption. Additional studies have suggested a role for fluoride in reducing cardiovascular disease. In a study of four towns in Finland, Luoma (1980) found that incidence of cardiovascular disease correlated negatively with water fluoride concentration. Taves (1978) likewise found that standard mortality ratios decreased to a greater extent in fluoridated cities from 1950 to 1970 as compared to non-fluoridated control cities. Both studies, however, relied on population-summary information for disease rates. A mechanism for this potential reduction in cardiovascular disease could be the ability of fluoride to inhibit the calcification of soft tissue such as the aorta, as demonstrated in *in vitro* studies (Taves and Neuman 1964; Zipkin et al. 1970).

About half of the male and female B6C3F_1 mice that died as a result of exposure to 67–71 mg fluoride/kg/day for 6 months as sodium fluoride in drinking water had mineralization of the myocardium (NTP 1990); some female mice also had myocardial degeneration.

Gastrointestinal Effects. The primary gastrointestinal effects following both acute and chronic oral exposure to fluoride consist of nausea, vomiting, and gastric pain. The irritation of the gastric mucosa is attributed to fluoride (as sodium fluoride) forming hydrofluoric acid in the acidic environment of the stomach (Hoffman et al. 1980; Waldbott 1981). The uncharged hydrogen fluoride molecule can then penetrate cell membranes and enter the neutral environment of the cytoplasm.

Thirty-four students (kindergarten through third grade) exhibited acute gastrointestinal effects after drinking water from school water fountains that provided a fluoride supplement designed to raise the water level to a range of 1–5 ppm (Hoffman et al. 1980). An accident with the delivery system resulted in the water levels reaching 375 ppm; specific doses could not be calculated, but were estimated to range from 1.4 to 90 mg per child. In another case, a 16-year-old girl vomited and had abdominal pain immediately after accidentally consuming 1 tablespoon of sodium fluoride (used as a dusting powder for poultry) (Rao et al. 1969).

Of the 150 cases involving fluoride intake reported to a poison control center from 1978 to 1979, most of the cases involved ingestion of <1 mg/kg fluoride, although exact doses could not be determined (Spoerke et al. 1980). Effects included nausea (13.9%), vomiting (77.8%), and diarrhea (8.3%). These effects usually subsided within 24 hours. Symptoms of a more serious nature were not reported.

Endoscopies were performed and biopsy samples were taken from 12 healthy volunteers either after no treatment (control) or 2 hours after drinking 20 mL of a solution containing 20 mg fluoride (1,000 ppm) as sodium fluoride (Spak et al. 1989). Both treatment and control tests were preceded by overnight fasts,

and at least 2 weeks were allowed between endoscopies, which allowed for healing of any iatrogenic injuries from the gastroscope. All subjects had six or more petechiae (minute hemorrhages) or erosions after fluoride treatment, while only one petechia or erosion was found in one control subject. Upon microscopic evaluation of biopsies, irritation of the stomach was found in all subjects after fluoride treatment, but none of the subjects showed stomach irritation after fasting only. Nausea was present in only one-third of the subjects, suggesting that nausea may not be the first sign of fluoride irritation of the gastric mucosa. The study suffers from several limitations. Only one dose was tested; the subjects were aware of whether or not they had received fluoride; it is unclear whether endoscopy videotapes were coded prior to evaluation; and the order of test and control endoscopies does not appear to have been randomized. For these reasons, this study has not been included in Table 3-3 or plotted in Figure 3-3.

While high levels of fluoride clearly can cause gastrointestinal irritation, it is unclear whether there are any gastrointestinal effects of chronic exposure to fluoride in drinking water. Gastrointestinal tract disorders were not evaluated in the Bartlett-Cameron study of the effect of water containing 8 ppm fluoride (Leone et al. 1954). The sole evidence of an effect comes from a study of twenty nonulcer dyspepsia patients at an outpatient clinic in India and 10 volunteers without gastrointestinal problems from the surgical clinic (Susheela et al. 1992). While none of the drinking water supplies of the controls had fluoride levels >1 ppm, the water supplies of 55% of the dyspepsia patients were at this level. In addition, all of the dyspepsia patients and 30% of the controls had serum fluoride levels >0.02 ppm (mean of the dyspepsia group, 0.1 ppm); all of the dyspepsia patients and none of the controls had urine fluoride levels >0.1 ppm (mean, 1.34 ppm). The study was compromised by small treatment size, undetermined total fluoride doses, undetermined nutritional status of the subjects, and lack of statistical comparisons. In addition, the appropriateness of the control population was not clear.

Seventy-eight workers engaged in the crushing and refining of cryolite, a mineral compound composed of sodium, aluminum, and fluoride, were examined (Moller and Gudjonsson 1932). Although an average exposure period was not presented, no workers with <2 years of exposure were included; 18 workers had been exposed for >10 years. Forty-two workers reported evidence of gastrointestinal effects. The primary effect was nausea, followed by loss of appetite and vomiting. Chronic indigestion was also reported in these workers. The study authors stated that the effects were due only to cryolite dust being swallowed (either due to dust being deposited in the mouth during mouth-breathing, or due to deposition on the bronchial tree followed by mucociliary action bringing the material to the epiglottis) and absorbed through the gastrointestinal tract. They based this conclusion on the fact that 21 enamel-, glass-, and sulphuric acid-industry workers exposed by inhalation to fluorine gas (some for up to 40 years) revealed no evidence of any effect on the stomach. In light of what is now known about the absorption of fluorides through the lung, the cryolite workers probably were exposed by both the oral and inhalation routes.

Decreased appetite, congestion of the duodenum, and mild diarrhea were reported in sheep given a single intragastric dose of 28.5 mg fluoride/kg in the form of sodium fluoride via nasoesophageal catheter (Kessabi et al. 1985). It is difficult to extrapolate possible human effects from this study because the gastrointestinal system of ruminants (sheep, cows, goats) is quite different from that of humans.

Thickening of the mucosa of the glandular stomach and punctate hemorrhages were seen in F344/N rats given 20 mg fluoride/kg/day as sodium fluoride in drinking water for 26 weeks (NTP 1990). Similar, but less severe, alterations were seen in some rats that received 7 mg fluoride/kg/day. Stomach ulcers were also seen in some high-dose males and females. Histologically identified stomach lesions included necrosis and hyperplasia. No gastrointestinal effects were reported in $B6C3F_1$ mice in this study at doses up to 67–71 mg fluoride/kg/day. No gastrointestinal effects were reported in the chronic portion of this study at doses up to 9.1 mg/kg/day (mice) or 4.5 mg/kg/day (rats). Roughened duodenal mucosa and a "cracked-clay" appearance of the absorptive cells was observed following daily dosage of nine rabbits with 5 mg/kg fluoride via oral gavage for 24 months (Susheela and Das 1988). The rabbit gastrointestinal system also differs from that of humans, and the study is limited by the small number of rabbits per group and the use of only one dose.

Hematological Effects. The incidence of abnormal white blood cell counts was significantly higher in Bartlett, Texas (8 ppm natural fluoride), than in Cameron, Texas (0.4 ppm fluoride). However, the study authors did not consider this finding as necessarily an effect of fluoride (Leone et al. 1954). No other significant hematological effects were observed.

As part of the 2-year NTP study of fluoride (NTP 1990), hematological analyses were conducted at 27 and 66 weeks. No treatment-related effects were observed at doses up to 4.5 and 9.1 mg/kg/day in F344/N rats and $B6C3F_1$ mice, respectively.

Lactating Holstein cows were fed a mineral supplement containing soft rock phosphate (6,000 ppm fluoride) and a protein supplement containing 1,088 ppm fluoride (Hillman et al. 1979). Because consumption of minerals fed *ad libitum* could not be determined accurately under farm conditions, no dose estimates could be made. After 9 months, red blood cells per unit volume, blood hemoglobin, hematocrit, and mean corpuscular volume were significantly lower ($p<0.05$) in herds exhibiting evidence of high fluoride exposure. The number of eosinophils increased with increasing urinary fluoride. Rabbits administered 4.52 mg fluoride/kg/day by gavage for 6–12 months had significantly decreased numbers of blood cells (e.g., erythrocytes, leukocytes, thrombocytes, monocytes, neutrophils) and hemoglobin (Susheela and Jain 1983). Similar, although not identical, results were seen in mice fed 5.2 mg fluoride/kg body weight (Pillai et al. 1988). These animals showed a significant decrease in red blood cell count, but a significant increase in white cells. Although a dose-effect relationship cannot be

determined from single-dose studies, these studies suggest that the hematopoietic system may be affected by oral exposure to fluoride.

Musculoskeletal Effects. Fluoride mottles teeth (dental fluorosis) when ingested in excess amounts during tooth development (1–8 years of age). During development of the deciduous and permanent teeth, excessive fluoride intake produces a malformation of the enamel surface, which then becomes stained (Hodge and Smith 1972). Fluoride causes mottled enamel by impairing the work of ameloblast cells (Hodge and Smith 1972).

Several different methods have been developed for quantifying dental fluorosis. Dean's index (Dean 1934) rates teeth as having class 0, no fluorosis; class 1, very mild (opaque white areas irregularly covering #25% of the tooth surface); class 2, mild (white areas covering 25–50% of the tooth surface); class 3, moderate (all surfaces affected, with some brown spots and marked wear on surfaces subject to attrition); and class 4, severe (widespread brown stains and pitting). People are classified according to the two most severely affected teeth; the mean fluorosis index is the mean of the score class. Other methods score tooth surfaces or relate fluorosis to the period during which the developing dentition could be exposed to fluoride. Drying teeth prior to scoring increases the frequency of observing opaque areas (DHHS 1991).

There is some evidence that levels of fluorosis have increased due to the multiple, widespread sources of fluoride in food processed with fluoridated water and dentifrices containing fluoride, in addition to the water of fluoridated communities. Comparison of fluorosis levels in the 21 cities with fluoride levels ranging from <0.4 to 2.7 ppm that were surveyed by Dean in the 1940s, and studies of dental fluorosis in 21 cities that were conducted in the 1980s found that both the prevalence and the severity of dental fluorosis were correlated with the level of fluoride in the drinking water (DHHS 1991). During this 40-year period, the prevalence of fluorosis in areas with <0.4 ppm fluoride increased from <1 to about 6%; nearly all of the increase was in the very mild and mild categories. Both the prevalence and severity of fluorosis increased in communities with 0.7–1.2 ppm fluoride, with prevalence increasing from about 13 to about 22%. Most of the increase was in the very mild and mild categories, which increased from 12.3 to 17.7%, and from 1.4 to 4.4% of the population, respectively. The combined prevalence of the severe and moderate categories increased from 0.0 to 0.9%. While there were some differences between the studies in the 1940s and those in the 1980s, such as the subject population and examination conditions, they do not affect the overall trends. Although total fluoride intake was not measured, these studies indicate that intake has increased since the 1940s, because fluorosis levels increased for all water fluoride levels.

Fluorosis levels in 1985 in communities with fluoride levels at about 1, 2, 3, and 4 ppm were compared with levels of fluorosis in the same communities in 1980 (Heifetz et al. 1988). Both examinations included 8–10-year-old and 13–15-year-old children. The 13–15-year-old children in the follow-up study had also participated in the initial study. While there were no marked changes in fluorosis levels in 8–10-year-old children, both the prevalence and severity increased in the 13–15-year-old children. Increases in the 1-ppm communities were mostly in the category of barely visible white spots. However, the percentage of labial surfaces of incisors and canines from children in the 2-ppm group that had brown mottling increased from 0 to 7.6%. Less marked increases in mottled and pitted teeth were seen in the higher dose groups. The increased levels of fluorosis were attributed to increased fluoride exposure from multiple sources.

While drinking water fluoride levels ranging from 0.7 to 3 ppm can reduce the incidence of dental caries, susceptibility to caries can increase at higher fluoride levels. Adolescents consuming water containing 5 ppm fluoride since birth were evaluated for fluorosis and prevalence of caries. The prevalence of dental fluorosis was 100%, with the 182 subjects showing effects ranging from mild to severe. The incidence of dental caries increased with increasing severity of fluorosis symptoms. The increase in caries was apparently caused by a degenerative effect of high levels of fluoride on ameloblast cells, resulting in porosity and hypoplasia of the tooth (Mann et al. 1987).

In an early study, 78 workers engaged in the crushing and refining of cryolite were examined. Thirty-nine workers showed evidence of skeletal fluorosis in the form of dense calcification in the long bones, cartilage, and in extreme cases, of the skull as well (Moller and Gudjonsson 1932). Although an average exposure period was not presented, no workers with <2 years of exposure were included; some workers had been exposed for as long as 40 years. The authors stated that the effects were due only to cryolite dust being swallowed and absorbed through the gastrointestinal tract. They based this conclusion on the fact that their examination of 21 workers exposed to fluorine gas (some for up to 40 years) revealed no skeletal effects. In light of what is now known about the absorption of fluorides through the lung, the cryolite workers probably incurred both oral and inhalation exposures.

Fluoride results in thickened bones and exostoses (skeletal fluorosis) when ingested in large doses for an extended period of time. Signs of skeletal fluorosis range from increased bone density to severe deformity, known as crippling skeletal fluorosis. Crippling fluorosis is characterized by complete rigidity of the spine, often accompanied by kyphosis (humpbacked) or lordosis (arched back). Reported cases are found almost exclusively in developing countries, particularly India, and are associated with malnutrition (Pandit et al. 1940). Tea consumption and high water intake due to the tropical climate are probably also contributing factors. As discussed in Chapter 6, tea is high in fluoride. High water intake would increase the intake of fluoride from water. It is generally stated that a dose of 20–80 mg/day (equivalent to 10 to

40 ppm in the water) is necessary for the development of crippling skeletal fluorosis (NAS 1971a), but individual variation, variation in nutritional status, and the difficulty of determining water fluoride levels in such situations make it difficult to determine the critical dose. Pandit et al. (1940) found severe skeletal fluorosis in people who had consumed 13–24 mg/day for >15 years. Cases of kyphosis, fused vertebrae, and marked exostoses (ossification of muscle attachments to bone) were reported in an area of India with water supplies containing 1.2–16.2 ppm fluoride (Singh et al. 1963), but fluoride levels were not reported for the water supplies used by the people with the most severe symptoms. Soil fluoride levels were not reported. Kyphosis, lordosis, and wedging of dorsal vertebra was reported in poorly nourished English children in 1932 (Kemp et al. 1942). Water supplies ranged from 0.3 to 1.2 ppm. The study was marked by small sample size and the absence of controls.

The incidence of early skeletal fluorosis in the United States is unknown, since it appears that the early signs can only be identified radiologically. A study of 116 people who had lived in an area with an average of 8 ppm fluoride in the drinking water for at least 15 years found a 10–15% incidence of fluoride-related bone changes (Leone et al. 1955). Coarsened trabeculation and thickened bone were observed, but no exostoses were evident, and the subjects were asymptomatic.

A limited number of cases of crippling skeletal fluorosis due to oral exposure have been reported in the United States. Where the doses are known, they are generally 15–20 mg fluoride/day for over 20 years; two of the cases were associated with renal disease, which would reduce fluoride excretion. Two of the cases were associated with drinking large quantities of water with >3.5 ppm fluoride. In the most severe case, a man who consumed at least 15 mg fluoride/day by drinking over 4 L of water with 3.5 ppm fluoride for 43 years developed kyphosis and severe joint stiffness. The diagnosis was confirmed by radiological analysis; bone fluoride content at autopsy was 6,100 ppm of dried bone (Sauerbrunn et al. 1965). Complete neck and spine rigidity was reported in a man who had ingested unspecified large volumes of water containing 4–7.8 ppm fluoride and large volumes of tea for 55 years (Goldman et al. 1971). A recent immigrant from Mexico developed symptoms consistent with spinal cord compression (Fisher et al. 1989). Her tap water in Mexico contained 3.9 ppm fluoride; fluoride levels in neighboring areas ranged from 0.1 to 5.5 ppm. A 40-year-old woman with renal failure developed progressive muscle weakness and severe pain in her ribs, back, and hip (Fisher et al. 1981). Fluoride content of an ashed iliac crest bone was 10,000 ppm. Questioning to determine her sources and intake of fluoride elicited the information that she practiced geophagia, the custom of eating earth, which is often a symptom of iron deficiency. The patient ingested about 15 mg fluoride/day, of which 10 mg/day was from eating soil, 4.2 mg/day from tea, and 1.4 mg/day from her drinking water, which had a fluoride content of 0.7 ppm. A 65-year-old woman who drank well water containing an undetermined fluoride concentration for most of her life developed paresthesias of both legs and pain in the back and chest (Bruns and Tytle 1988). The paresthesias were considered secondary to bone deformities. The fluoride level in the iliac crest bone

was reported as 1,900 ng/L (sic) (normal is <140 ng/L); urinary fluoride was 3.39 mg/L (normal, 0.2–1.0 mg/L). Two of the cases were initially diagnosed as bone malignancies, but were recognized as fluorosis upon further investigation (Bruns and Tytle 1988; Fisher et al. 1981).

Fluoride is found in all bone, with the concentration depending on total fluoride exposure. The amount varies among different bones. Levels of fluoride in human bone are generally determined by biopsy of the iliac crest bone, and are generally reported as ppm of bone ash. Average bone contains 500–1,000 ppm fluoride (Boivin et al. 1988; Franke et al. 1975). Bone from people with preclinical skeletal fluorosis, which is generally asymptomatic and characterized by slight radiologically detectable increases in bone mass, contains 3,500–5,500 ppm fluoride. Sporadic pain, joint stiffness, and osteosclerosis of the pelvis are observed at 6,000–7,000 ppm, while chronic joint pain, increased osteosclerosis, and slight calcification of ligaments occur at 7,500–9,000 ppm. Crippling fluorosis is observed at fluoride bone concentrations >10,000 ppm (Franke et al. 1975). The fluoride concentration in bone increases with age (Zipkin et al. 1958). In a group of five people ages 64–85 who had lived for at least 10 years in an area with water containing 1 ppm fluoride, the average fluoride concentration of the iliac crest bone was 2,250 ppm of bone ash.

Since fluoride increases bone density, it has been hypothesized that fluoride could be used to treat osteoporosis. Additional support for this hypothesis came from a study that found that women in a high-fluoride area (4–5.8 ppm fluoride in the water) had lower incidences of decreased bone density and collapsed vertebrae than did women in a low-fluoride area (0.15–0.3 ppm) (Bernstein et al. 1966). However, there is evidence that the newly formed bone following fluoride treatment may be more brittle and more fracture-prone. The bones of a man with severe skeletal fluorosis had increased compressive strength, but decreased tensile strength and modulus of elasticity (a measure of stiffness, or resistance to being strained by a load) compared to normal controls (Evans and Wood 1976). However, only one subject was tested, and the fact that he had been bedridden for the previous 5 years may have been a confounding factor.

Numerous studies have examined the possible relationship between exposure to fluoride in drinking water and the risk of bone fractures. Many of these studies are ecological studies that examined communities with high level of fluoride in the water or fluoridated water (Arnala et al. 1984; Cooper et al. 1990, 1991; Danielson et al. 1992; Jacobsen et al. 1990; Kröger et al. 1994; Madans et al. 1983; Simonen and Laittenen 1985; Sowers et al. 1986); a few prospective (Cauley et al. 1995; Phipps et al. 2000) or retrospective (Kurttio et al. 1999) studies have also examined this possible association. These studies have found conflicting results, with studies finding a lower or higher incidence of hip fractures or no differences in hip fracture between humans exposed to fluoride in drinking water.

Several studies have found decreases in hip fracture incidences in communities with fluoride in the drinking water, suggesting that there may be a beneficial effect. Simonen and Laittinen (1985) examined male and female residents older than 50 years living in two cities in Finland with either trace amounts of fluoride in the water or with 1 ppm fluoride in the water. The occurrence of femoral neck fractures was lower in the men 50–80 years old and women >70 years old living in the area with fluoridated water, as compared to the low fluoride community. No difference in femoral neck fracture was observed in women 50–69 years of age. Madans et al. (1983) examined the association between fluoride in drinking water and risk of hip fractures using hip fracture data from the National Health Interview Surveys of 1973–1977 and Centers for Disease Control and Prevention (CDC) data on the percent of a population in each U.S. count served with water having a natural or adjusted fluoride content of at least 0.7 ppm in 1973. Female residents over 45 years of age living in areas with lower fluoride levels in the drinking water had 9% more hip fractures than women living in high fluoride areas; however, the difference was not statistically significant. In a prospective study of older women, Phipps et al. (2000) examined the possible relationship between living in an area with fluoridated water and the risk of fractures. Higher bone mineral density of the lumber spine and femoral neck and trochanter and lower bone mineral density of the radius were observed in women continuously living in an area with fluoridated water, as compared to residents in a non-fluoridated water area. Fewer spine, hip, and humerus fractures were also observed in this group. However, a higher incidence of wrist fractures were also observed in the continuous exposure group. Cauley et al. (1995) examined a subset of this population, and found no effect on age-adjusted axial and appendicular bone mineral density and no effect on the risk of vertebral or nonvertebral fractures.

In contrast to the results of these studies, other studies have found an increase in the incidence of hip fractures in communities with fluoride in the drinking water. Sowers et al. (1986) examined female residents living in three communities in northwest Iowa with either high fluoride (4 mg/L)-low calcium (14–19 mg/L), low fluoride (1 mg/L)-high calcium (336–390 mg/L), or low fluoride (1 mg/L)-low calcium (62–71 mg/L) levels in the drinking water. The subjects had lived in the communities for at least 5 years and did not have wrist or forearm fractures in the previous 2 years. Among women 55–80 years old living in the high fluoride community, bone mass of the radius was significantly lower and a higher incidence of hip fractures was observed, as compared to the other groups. No effect was seen in younger women (20–35 years old). A geographical correlational study of 541,985 white women hospitalized for hip fractures found a weak association (regression coefficient=0.001, p=0.1) between hip fracture incidence and fluoridation of water (Jacobsen et al. 1990). The association was strengthened (regression coefficient=0.003, p=0.0009) after correcting by county for other factors found to correlate with hip fracture incidence (latitude, hours of sunlight, water hardness, income level, and percentage of land in farms).

A study in England and Wales also found increased rates of hip fractures in men and women over age 45 as water fluoride levels increased up to 0.93 ppm (Cooper et al. 1991). Hip fracture rates in 39 counties (standardized by age and sex) were compared with water fluoride levels in those counties. In the original analysis (Cooper et al. 1990), no significant correlation was found. However, when the authors reanalyzed the data using a weighted least-squares technique (weighting by the size of the population aged $45 years) to account for differences in the precision of the county-specific rates, a significant positive correlation between water fluoride levels and hip fracture rates was found ($r=0.41$, $p=0.009$). The correlation existed for both women ($r=0.39$, $p=0.014$) and men ($r=0.42$, $p=0.007$) (Cooper et al. 1991). Kurttio et al. (1999) studied over 144,000 residents living in rural areas of Finland from 1967–1980. When all age groups were considered together, no relationship between fluoride levels in drinking water and the risk of hip fractures was found. However, among women aged 50–64 years with higher fluoride levels, an increase in the risk of hip fractures was found. No consistent relationships were found in men or in older women. The study authors suggested that other risk factors for hip fracture may be more important than fluoride exposure in determining risk of hip fractures in older women. An ecologic cohort study compared the hip fracture rate for men and women in a Utah community that had water fluoridated to 1 ppm with the rate in two communities with water containing <0.3 ppm fluoride (Danielson et al. 1992). Fluoridation began in the fluoridated community in 1966. The age-adjusted rate was significantly elevated in both women (relative risk 1.27, 95% confidence interval 1.08–1.46) and men (relative risk 1.41, 95% confidence interval 1.00–1.81). In men, the rates in the fluoridated and nonfluoridated communities were similar until age 70. From age 75 on, the difference between the rates in the fluoridated and nonfluoridated areas increased with age. The difference between the hip fracture rates in the fluoridated and nonfluoridated areas increased for women in the 70- and 75-year age groups. However, the fracture rates in women at ages $80 years old were similar in the fluoridated and nonfluoridated towns. The study authors attributed this to the fact that women older than 80 years would have already gone through menopause by the beginning of fluoridation, and so would have had less bone remodeling and less incorporation of fluoride into the bone. The study authors also suggested that the reason that they found an effect when other investigators have not was the low levels of exposure to risk factors for osteoporosis (smoking and alcohol) in the Utah populations. This was a well-conducted study that suggests that communities with fluoridated water have an elevated risk of hip fracture. However, several possible confounding factors were not examined. Calcium levels in the water, total calcium and vitamin D intake, and individual fluoride intake were not determined. Estrogen use was not evaluated, but was assumed to be similar since the communities were similar distances from larger medical centers. In addition, estrogen levels would not cause the effect in men.

Other studies have not found a relationship between fluoride in drinking water and hip fracture prevalence. No significant differences in the incidence or type of upper femoral fracture were observed when groups of subjects living in communities with low fluoride (<0.3 ppm), fluoridated (1.0–1.2 ppm),

or high fluoride (>1.5 ppm) drinking water (Arnala et al. 1986). An increase in the fluoride content of bone and an increase in the volumetric density of the osteod were observed in the residents in the high fluoride area, as compared to the low fluoride area. Kröger et al. (1994) found no effect on self-reported fractures among a group of older Finnish residents (mean age of approximately 53 years) living in an area with fluoridated water (1.0–1.2 mg/L), as compared to residents living in an area with low fluoride levels in the drinking water (<0.3 ppm). Increases in spine and femoral neck bone mineral density were observed in the fluoridated water group.

Because of the increased levels of bone mineral density observed in many studies of residents exposed to fluoride in drinking water, fluoride has been used as a treatment for osteoporosis. A number of studies have examined the efficacy of this treatment. A prospective, randomized, double-blind, placebo-controlled study of 202 women with postmenopausal osteoporosis ascertained the effect of administering 34 mg fluoride/day as sodium fluoride (0.56 mg fluoride/kg/day) (Riggs et al. 1990). Both groups received 1,500 mg calcium/day. Rigorous criteria excluded patients with metabolic diseases. A total of 135 patients (66 in the treatment group and 69 in the control group) completed the full 4 years of treatment. Although bone mineral density in the lumbar spine, femoral neck, and femoral trochanter increased markedly in the treatment group, bone mineral density in the shaft of the radius decreased 4%. There was no significant difference in the number of new vertebral fractures between the treatment and control groups, although the number of vertebral fractures in the fluoride group was slightly elevated in the first year. In contrast, the level of nonvertebral fractures in the fluoride group was 3.2 times that of the control group, with significant increases in both the frequency and rate of fractures. Most of the increase was due to increased incidences of incomplete ("stress") fractures, which occurred 16.8 times more often in the treatment group. In a follow-up to this study, Riggs et al. (1994) examined 50 of the women in the fluoride treatment group after an additional2 years of treatment with 34 mg fluoride/day as sodium fluoride. The lumbar spine, femoral neck, and femoral trochanter bone mineral density continued to increase and the bone mineral density of the radium continued to decrease during years 4–6 of treatment. The vertebral fracture rate decreased during years 4–6 as compared to years 0–4. The nonvertebral fracture rate also decreased during the last 2 years, but the rate for the full 6-year period was still 3 times higher than the rate in the placebo control group. In addition to extending the study for an additional 2 years, Riggs et al. (1994) also re-examined the data from the previous study. Vertebral fracture rate was influenced by several factors. Vertebral fracture rate decreased with increasing lumbar spine bone mineral density except in the cases where the higher bone mineral density was associated with a rapid rate of increase in the lumbar spine bone mineral density or a large increase from baseline serum fluoride level.

In a similar study by Kleerekoper et al. (1991), the anti-fracture efficacy of 34 mg fluoride/day as sodium fluoride was examined in 46 postmenopausal women (mean age of 66.2 years) with spinal osteoporosis.

A daily dose of 1,500 mg calcium was also administered to this group as well as a placebo control group of 38 postmenopausal women with spinal osteoporosis (mean age of 67.9 years). No significant differences in bone mineral density of the forearm, vertebral fractures, or peripheral fractures were found. A significant increase in painful lower extremity syndrome was observed in the fluoride group. It should be noted that Riggs et al. (1990, 1994) considered the lower extremity syndrome to be incomplete fractures and the incidence of incomplete fractures was added to the complete fracture incidence to calculate nonvertebral fracture incidence.

Haguenauer et al. (2000) performed a meta-analysis to examine the effects of fluoride on the treatment and prevention of post-menopausal osteoporosis using the data from the Riggs et al. (1990, 1994), Kleerekoper et al. (1991), and 10 other studies. The meta-analysis showed a significant increase in bone mineral density in the lumbar spine and hip and a decrease in bone mineral density in the forearm after 2 or 4 years of fluoride treatment. When the data from all studies was used, fluoride treatment for 2 or 4 years did not affect the relative risk of vertebral fractures. However, in studies in which the subjects were exposed to low levels of fluoride or a slow-release formulation for 4 years, a significant decrease in vertebral fracture relative risk was seen. An increase in the relative risk of nonvertebral fracture was observed when data from all studies were used; no effect was seen in studies using low levels of fluoride (<30 mg/day) or slow-release fluoride.

Evidence from animal experiments supports the association of high levels of fluoride and adverse effects on bone. The femurs of weanling male rats of a Wistar-derived strain that were given $9.5 mg fluoride/kg/day as sodium fluoride for 2 weeks exhibited a marked decrease in the modulus of elasticity. It is not clear if the change was analyzed statistically. No lower doses were tested (Guggenheim et al. 1976). Musculoskeletal effects in albino rats (strain not identified) following oral exposure of intermediate duration have been investigated. After 30 days of exposure to 100 ppm of fluoride in water (14 mg/kg), tibia bones were broken and allowed to heal (Uslu 1983). Collagen synthesis was determined to be defective, and fracture healing was delayed, when compared to the controls. Decreased bone growth and signs of fluorosis were observed in rats given 19 mg/kg in their drinking water and adequate calcium for 5 weeks; with elevated calcium levels, fluorosis was not observed until the fluoride level reached 35 mg/kg (Harrison et al. 1984). Rats administered 10.5 mg fluoride/kg/day for 5 weeks had significantly decreased mineral content and increased proline content of tooth enamel (DenBesten and Crenshaw 1984). According to the authors, chronic high levels of fluoride interfere with the progressive deposition of mineral and withdrawal of organic matrix and water that characterizes normal preeruptive enamel development. Male mice administered 0.80 mg fluoride/kg/day for 4 weeks exhibited a statistically significant increase in the bone formation rate and a slight but statistically significant decrease in bone calcium levels (Marie and Hott 1986). The authors concluded that 0.80 mg fluoride/kg increased the population of osteoblasts under the conditions of this experiment. Turner et al. (1992) found a

biphasic relationship between bone strength and bone fluoride content in rats. At lower fluoride intakes, increased bone strength was observed; the maximum bone strength was achieved at 1,000–1,500 ppm fluoride in the bone. At fluoride concentrations higher than 1,000 ppm, bone strength started to decrease.

It is possible that the decreased level of bone resorption in the presence of fluoride, and the associated lowered serum calcium levels, would lead to secondary hyperparathyroidism in an attempt to maintain normocalcemia. To address this issue, rats were dosed with 3.3 mg fluoride/kg in drinking water for 46 weeks (Rosenquist et al. 1983). There were no changes in serum calcium or parathyroid hormone levels, and no increase in parathyroid activity.

The sagittal crests were enlarged and/or deformed in three of six adult female mink fed 9.1 mg fluoride/kg/day as sodium fluoride for 382 days (Aulerich et al. 1987). The authors attributed the abnormalities of the sagittal crests to increased osteoblastic activity. After about 210 days of exposure, the females were mated. The mink kits were exposed during gestation and during the suckling period, and were fed the same diets as their mothers. Kits in the 5.0 mg fluoride/kg/day and over groups had dark mottling of their teeth. Several of the kits had broken canines.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to fluorine, hydrogen fluoride, or fluoride.

Pale, granular hepatocytes, compatible with parenchymal degeneration, were observed in mice administered 0.95 mg fluoride/kg/day in drinking water for 7–280 days (Greenberg 1982a). Fatty granules were observed after 3 weeks. Liver congestion was observed in sheep given a single intragastric dose of fluoride as low as 9.5 mg fluoride/kg. Mild serum increases of liver enzymes (glutamate dehydrogenase [GDH] and gamma-glutamyl transferase [GGT]) also occurred in sheep administered 38 mg fluoride/kg (Kessabi et al. 1985). It is difficult to use this result to predict to possible human effects because ruminants (sheep, cows, goats) have gastrointestinal systems quite different from that of humans.

Enlarged liver cells with multiple foci were seen in about half of the male B6C3F_1 mice that died after receiving 33–36 or 67–71 mg fluoride/kg/day for up to 6 months as sodium fluoride in drinking water (NTP 1990). This change was seen in all of the female mice that died at the 71 mg/kg/day dose level. No liver effects were seen in a parallel experiment with F344/N rats at doses up to 20 mg/kg/day. Similarly, no liver histopathology was seen in the chronic portion of this study (NTP 1990), in which rats received total fluoride doses (amount added to water plus endogenous fluoride in food) of about 4.5 mg/kg/day (rats) or up to 9.1 mg/kg/day (mice). Alkaline phosphatase levels were significantly increased in male and female mice at the 66-week interim sacrifice of the chronic study.

Renal Effects. One study was located in which ingestion of fluoride appeared to be linked with renal insufficiency (Lantz et al. 1987). A 32-year-old man ingested 2–4 L of Vichy water (a highly gaseous mineral water containing sodium bicarbonate and approximately 8.5 mg/L of fluoride) every day for about 21 years. This exposure ended 4 years before his hospital admission. The patient also had osteosclerosis and a moderate increase in blood and urinary levels of fluoride. No teeth mottling was observed. The authors could not find factors, other than fluoride, related to his interstitial nephritis. No effect on the incidence of urinary tract calculi or the incidence of albuminuria was found in the Bartlett-Cameron study of people drinking water containing 8 ppm fluoride (Leone et al. 1954).

Congestion of the kidney was observed in sheep given a single intragastric dose of fluoride as low as 9.5 mg fluoride/kg (Kessabi et al. 1985). An intermediate exposure study tested the effect of administering up to 67–71 mg fluoride/kg/day to $B6C3F_1$ mice (8–9/group) as sodium fluoride in drinking water for 26 weeks (NTP 1990). Acute nephrosis characterized by extensive multifocal degeneration and necrosis of the tubular epithelium was believed to be the main cause of death in two of the four males exposed to 67 mg/kg/day that died, the single male that died after exposure to 33 mg/kg/day, and two of the four females in the high dose group that died. No kidney histopathology was observed in surviving mice or in rats exposed to 20 mg fluoride/kg/day and higher (NTP 1990).

Changes in kidney histology were seen in mature Swiss mice given a dose of sodium fluoride in drinking water for up to 280 days that was described as the maximum dose that could be chronically tolerated, i.e., 1.9 mg/kg/day (Greenberg 1986). Using a sensitive staining technique, increased collagen levels were seen after about 45 days. Thickening of the Bowman's capsule, edematous swelling of the tubules, and infiltrations of mononuclear cells were also noticed. No kidney pathology was seen in a 2-year study in $B6C3F_1$ mice at doses up to 8.1 mg/kg/day (males) or 9.1 mg/kg/day (females), or in F344/N rats at doses up to 4.1 mg/kg/day (males) or 4.5 mg/kg/day (females) (NTP 1990).

Endocrine Effects. Significant increases in serum thyroxine levels were observed in residents of North Gujarat, India with high levels of fluoride in the drinking water (range of 1.0–6.53 mg/L; mean of 2.70 mg/L) (Michael et al. 1996). No significant changes in serum triiodothyronine or thyroid stimulating hormone levels were found. Increases in serum epinephrine and norepinephrine levels were also observed. It is unclear if nutritional deficiencies played a contributing role to the observed endocrine effects.

Fluoride has been shown to affect the endocrine system in rats given 0.5 mg fluoride/kg/day as sodium fluoride in drinking water every day for 2 months (Bobek et al. 1976). These animals showed decreased thyroxine levels and an increased T_3-resin uptake ratio.

It is possible that the decreased level of bone resorption in the presence of fluoride, and the associated lowered serum calcium levels, would lead to secondary hyperparathyroidism in an attempt to maintain normocalcemia. To address this issue, rats were dosed with 3.3 mg fluoride/kg in drinking water for 46 weeks (Rosenquist et al. 1983). There were no changes in serum calcium or parathyroid hormone levels, and no increase in parathyroid activity.

Body Weight Effects. Final body weight was reduced by >40% relative to the controls in female F344/N rats administered 25 mg fluoride/kg/day as sodium fluoride in drinking water for 14 days; body weight in males was reduced by >10% at doses $6.3 mg/kg/day (NTP 1990). A clear and consistent effect on body weight of $B6C3F_1$ mice was seen only at the high dose (69 mg/kg/day), which was lethal to males (3/5), but not to females. In the intermediate-duration (6 month) phase of the study, the body weight of mice administered 17 mg fluoride/kg/day was reduced by 20%; it was reduced by 10% at 19 mg/kg/day in male and female rats.

F344/N rats and $B6C3F_1$ mice given large doses of sodium fluoride in drinking water for 14 days had reduced water intake (NTP 1990). Male and female rats given 25 mg fluoride/kg/day drank about 30% less water than the controls. Water consumption by male rats given 51 mg fluoride/kg/day was 50% of controls, while it was 25% of controls for females. Similarly, mice given 69 mg fluoride/kg/day drank #60% the volume of water consumed by the controls. This means that actual fluoride doses are lower than the estimates given here, since these values were calculated assuming normal water intake. However, the reduced water intake may have been due to the disagreeable taste of fluoride at high concentrations in the water.

3.2.2.3 Immunological and Lymphoreticular Effects

A request to the American Academy of Allergy was made by the U.S. Public Health Service for an evaluation of suspected allergic reactions to fluoride as used in the fluoridation of community water supplies (Austen et al. 1971). The response to this request included a review of clinical reports and an opinion as to whether these reports constituted valid evidence of a hypersensitivity reaction to fluoride exposure of types I, II, III, or IV (Austen et al. 1971), which are, respectively, anaphylactic or reaginic, cytotoxic, toxic complex, and delayed-type reactivity. The Academy reviewed the wide variety of symptoms presented (vomiting, abdominal pain, headaches, scotomata [blind, or partially blind areas in the visual field], personality change, muscular weakness, painful numbness in extremities, joint pain, migraine headaches, dryness in the mouth, oral ulcers, convulsions, mental deterioration, colitis, pelvic hemorrhages, urticaria, nasal congestion, skin rashes, epigastric distress, and hematemesis) and concluded that none of these symptoms were likely to be immunologically mediated reactions of types I–IV. No

studies were located that investigated alterations in immune response following fluoride exposure in humans.

In a study with rabbits administered 4.5 mg fluoride/kg/day as sodium fluoride for 18 months, decreased antibody titers were observed (Jain and Susheela 1987). These results were observed after 6 months of treatment; the authors hypothesized that a threshold level is reached at which time the immune system is impaired. However, as only one dose level (4.5 mg fluoride/kg/day) was tested, no dose-effect relationships can be established.

3.2.2.4 Neurological Effects

Fluoride has been shown to interfere with glycolysis. (See Section 3.4 for a discussion of the effect of fluoride on various glycolytic enzymes.) Because the central nervous system relies heavily on this energy source, hypotheses have been advanced as to a mechanism for fluoride effects on the central nervous system. Although effects on glycolytic enzymes could explain the neuromuscular symptoms seen frequently in cases of fluoride poisoning (e.g., tetany, paresthesia, paresis, convulsions), studies tend to indicate that hypocalcemia caused by fluoride binding of calcium causes these symptoms (Eichler et al. 1982). As discussed in the Developmental Effects section, decreases in intelligence were reported in children living in areas of China with high levels of fluoride in the drinking water, as compared to matched groups of children living in areas with low levels of fluoride in the drinking water (Li et al. 1995a; Lu et al. 2000), but these studies are weak inasmuch as they do not address important confounding factors.

There are limited animal data on the neurotoxicity of fluoride. Significant decreases in spontaneous motor activity was observed in rats exposed via gavage to 9 mg fluoride/kg/day as sodium fluoride in saline for 60 days (Paul et al. 1998). No alterations in motor coordination, as assessed with the rotarod test, were found. A decrease in blood cholinesterase activity was also observed in these rats. Another study (Mullenix et al. 1995) found alterations in spontaneous behavior in female rats exposed to 7.5 mg fluoride/kg/day as sodium fluoride in drinking water for 6 weeks beginning at 3 weeks of age and in female rats exposed to 6.0 mg fluoride/kg/day as sodium fluoride in drinking water for 6 weeks beginning at 13 weeks of age. The study authors noted that the observed effects were consistent with hyperactivity and cognitive deficits. Another study found increases in the frequency of neuronal abnormalities in the neocortex and a bilateral accumulation of β-amyloid in the thalamus of rats exposed to 0.11 mg fluoride/kg/day as sodium fluoride in drinking water (Varner et al. 1998). This study did not assess neurofunction; thus, it is difficult to assesses the toxicological significance of these effects.

3.2.2.5 Reproductive Effects

There are limited data on the potential of fluoride to induce reproductive effects in humans following oral exposure. A meta-analysis found a statistically significant association between decreasing total fertility rate and increasing fluoride levels in municipal drinking water (Freni 1994). Annual county birth data (obtained from the National Center for Health Statistics) for over 525,000 women aged 10–49 years living in areas with high fluoride levels in community drinking water were compared to a control population approximately 985,000 women) living in adjacent counties with low fluoride drinking water levels. The fluoride-exposed population lived in counties reporting a fluoride level of 3 ppm or higher in at least one system. The weighted mean fluoride concentration (county mean fluoride level weighted by the 1980 size of the population served by the water system) was 1.51 ppm (approximately 0.04 mg fluoride/kg/day), and 10.40% of the population was served by water systems with at least 3 ppm fluoride. The mean weighted mean fluoride concentration in the control population was 1.08 ppm (approximately 0.03 mg fluoride/kg/day). However, this meta-analysis relied on a comparison of two quite disparate data sets, inasmuch as the fluoridation population often did not correlate well with the population for whom health statistics was available. Furthermore, other studies have not found a similar correlation. Another study found significantly decreased serum testosterone levels in 30 men diagnosed with skeletal fluorosis and in 16 men related to men with fluorosis and living in the same house as the patient (Susheela and Jethanandani 1996). The mean drinking water fluoride levels were 3.9 ppm (approximately 0.11 mg fluoride/kg/day), 4.5 ppm (0.13 mg fluoride/kg/day), and 0.5 ppm (0.014 mg fluoride/kg/day) in the patients with skeletal fluorosis, related men, and a control group of 26 men living in areas with low endemic fluoride levels. No correlations between serum testosterone and urinary fluoride levels or serum testosterone and serum fluoride levels were found. One limitation of this study is that the control men were younger (28.7 years) than the men with skeletal fluorosis (39.6 years) and the related men (38.7 years). In addition, the groups are small and potentially confounding factors are not well addressed.

Studies that reported an increased incidence of Down's syndrome in areas of high fluoridation have not been replicated by several other investigations (Berry 1958; Erickson et al. 1976; Needleman et al. 1974). No correlation was found between fluoridation and Down's syndrome incidence (corrected for maternal age) in a study of over 234,000 children in fluoridated areas and over 1,000,000 children in low-fluoride areas (Erickson et al. 1976). Ascertainment was based on birth certificates and hospital records, but was probably incomplete. Ascertainment was nearly complete in a study of over 80,000 children in fluoride areas and over 1,700,00 in low-fluoride areas, but no age-specific rates were reported (Needleman et al. 1974). Similarly, a study of the incidence of Down's syndrome in England did not find an association with the level of fluoride in water, but age-specific rates were not determined and tea was not taken into account as a source of fluoride (Berry 1958).

Animal studies have examined the effect of fluoride on reproductive hormone levels, histology of the testes, spermatogenesis, and fertility. No alterations in mean serum levels of testosterone, luteinizing hormone, or follicle stimulating hormone were found in male rats exposed to 16 mg fluoride/kg/day as sodium fluoride in drinking water for 14 weeks or in their male offsprings exposed during gestation, lactation, and for 14 weeks after weaning (Sprando et al. 1997). In contrast, significant decreases in serum testosterone levels were observed in rats receiving daily gavage doses of 4.5 mg fluoride/kg/day as sodium fluoride for 50 days (Narayana and Chinoy 1994) and in rats exposed for 60 days to 4.5 mg fluoride/kg/day as sodium fluoride in the diet (Araibi et al. 1989).

No alterations in Sertoli cells or in the seminiferous tubules were observed in the male offspring of rats exposed during gestation, lactation, and for 14 weeks post weaning to 16 mg fluoride/kg/day as sodium fluoride in drinking water (Sprando et al. 1998). However, other studies have reported testicular damage, which appears to be directly related to the length of exposure. No histological alterations were observed in the testes of rats exposed to 21 mg fluoride/kg/day as sodium fluoride for 6 weeks (Krasowska and Wlostowski 1992). However, after 16 weeks of exposure, seminiferous tubule atrophy was observed at 7.5 mg fluoride/kg/day and higher (Krasowska and Wlostowski 1992). A decrease in the mean diameter of the seminiferous tubules was observed in rats exposed to 2.3 or 4.5 mg fluoride/kg/day as sodium fluoride in the diet for 60 days (Araibi et al. 1989); thickening of the peritubular membrane of the seminiferous tubules was also observed at 4.5 mg fluoride/kg/day. Consistent with the decreases in serum testosterone levels, significant decreases in Leydig cell diameter were observed in rats (Narayana and Chinoy 1994) and rabbits (Susheela and Kumar 1991) receiving 4.5 mg fluoride/kg/day via gavage as sodium fluoride in water for 50 days or 18–23 months, respectively.

Although some studies have not found significant alterations in spermatogenesis or sperm morphology, a number of studies have reported adverse effects. No alterations in sperm head abnormalities (Li et al. 1987a) or sperm morphology (Dunipace et al. 1989) were observed in $B6C3F_1$ mice administered sodium fluoride by gavage at doses up to 32 mg fluoride/kg/day for 5 days and killed 30 days later or in $B6C3F_1$ mice administered 23 mg fluoride/kg/day as sodium fluoride in water. In CD rats administered 4.5 mg fluoride/kg/day as sodium fluoride in the diet for 60 days, a significant decrease in the percentage of seminiferous tubules containing spermatozoa was observed (Araibi et al. 1989). Damage to the spermatid and epididymal spermatozoa were observed in rabbits administered by gavage 4.5 mg fluoride/kg/day as sodium fluoride in water for at least 18 months (Kumar and Susheela 1994, 1995), and complete cessation of spermatogenesis was observed after 29 months of exposure (Susheela and Kumar 1991). A number of studies have found significant alterations in cauda epididymal and vas deferens sperm. Decreased sperm counts, sperm motility, and sperm viability (the ratio of live to dead sperm) have been observed in rats exposed to 2.3 mg fluoride/kg/day and higher (Chinoy et al. 1992, 1995) and mice (Chinoy and Sequeira 1992) and guinea pigs (Chinoy et al. 1997) exposed to 4.5 mg fluoride/kg/day and

higher. When exposed male rats were mated with unexposed males, decreased fertility was observed at 2.3 mg fluoride/kg/day as sodium fluoride and higher (Chinoy and Sequeira 1992; Chinoy et al. 1992). The alterations in sperm and the infertility were reversible 30–60 days after termination of a 30-day exposure period (Chinoy and Sequeira 1992).

Adverse reproductive effects have also been observed in females. Nearly complete infertility was observed in female Swiss-Webster mice exposed to 19 mg fluoride/kg/day as sodium fluoride in the drinking water for 25 weeks (Messer et al. 1973). However, this effect was not repeated in another study of Webster mice exposed to 13 mg fluoride/kg/day as sodium fluoride in the diet for three generations (Tao and Suttie 1976). The study authors attributed the difference between this study and the Messer et al. (1973) study to the higher iron levels in the Tao and Suttie (1976) study, as anemia was reported by Messer et al. (1973), but not by Tao and Suttie (1976). Decreased estrus rate and increased incidence of missed pregnancies was observed in Sheltie dogs fed dog food with supplemented with rock phosphate at a level of 11.5 mg fluoride/kg/day (Shellenberg et al. 1990). However, these changes were also observed in groups provided with distilled water rather than well water. No adverse effects on reproduction were observed in a two-generation rat study in which male and female Upj TUC(SD)spf rats were fed diets containing 23 mg fluoride/kg/day (Marks et al. 1984). Additional evidence that fluoride adversely affects female reproduction includes decreased lactation in rats exposed to 21 mg fluoride/kg/day in drinking water for 88 days (Yuan et al. 1994) and decreased calving rate (Van Rensburg and de Vos 1966) and decreased milk production (Maylin and Krook 1982) in cows ingesting large amounts of fluoride.

The highest NOAEL values and all reliable LOAEL values for reproductive effects for each species and duration category are recorded in Table 3-3 and plotted in Figure 3-3.

3.2.2.6 Developmental Effects

Fluoride crosses the placenta in limited amounts and is found in fetal and placental tissue (Gedalia et al. 1961; Theuer et al. 1971). The available human data suggest that fluoride has the potential to be developmentally toxic at doses associated with moderate to severe fluorosis. The human and animal data suggest that the developing fetus is not a sensitive target of fluoride toxicity.

Analysis of birth certificates and hospital records for over 200,000 babies born in an area with fluoridated water and over 1,000,000 babies born in a low fluoride area found no difference in the incidence of birth defects attributable to fluoride (Erickson et al. 1976). Exposure to high levels of fluoride has been described together with an increased incidence of spina bifida (Gupta et al. 1995). The occurrence of spina bifida was examined in a group of 50 children aged 5–12 years living in an area of India with high levels of fluoride in the drinking water (4.5–8.5 ppm) and manifesting either clinical (bone and joint pain,

stiffness, and rigidity), dental, or skeletal fluorosis. An age- and weight-matched group of children living in areas with lower fluoride levels (#1.5 ppm) served as a control group. Spina bifida was found in 22 (44%) of the children in the high fluoride area and in six (12%) children in the control group. This study did not examine the possible role of potentially important nutrients such as folic acid, however, and had other study design flaws.

A study by Li et al. (1995a) examined intelligence in children living in areas with high fluoride levels due to soot from coal burning. A group of 907 children aged 8–13 years were divided into four groups depending on the existence and severity of dental fluorosis; 20–24 children in each age group for each area were examined for intelligence. A significant decrease in IQ was measured in children living in the medium- (mean IQ of 79.7) and severe- (mean of 80.3) fluorosis areas, as compared to the children living in the non- (mean of 89.9) or slight- (mean of 89.7) fluorosis areas. More children with IQs of <70 and 70–79 and fewer children with IQs of 90–109 and 110–119 were found in the medium- and severe-fluorosis areas than in the non- or slight-fluorosis areas. No information on exposure levels were provided; the mean urinary fluoride levels were 1.02, 1.81, 2.01, and 2.69 mg/L in the non-, slight-, medium-, and severe-fluorosis areas, respectively. Numerous potentially confounding variables were not mentioned in this study, however, which raises questions regarding the validity of the study's findings. A study by Lu et al. (2000) also examined exposure to high fluoride levels and decreased intelligence. Sixty children aged 10–12 years living in an area with high fluoride levels in the drinking water (3.15 mg/L) were examined for intelligence. The test results were compared to a group of 58 children with similar social, education, and economic backgrounds who lived in an area with low fluoride levels in water (0.37 mg/L). A significant decrease in IQ was observed in the high fluoride area (mean IQ of 92.27) as compared to the control group (103.05). Additionally, there was a significantly higher number of children from the high exposure area with IQ scores of <70 (retarded) and 70–79 (borderline retarded) than in the control group. A significant inverse relationship between urinary fluoride levels and IQ was also found. Nevertheless, because this study relied on small groups and presented scant discussion of numerous potential confounders, the strength of its conclusions are questionable.

No alterations in the number of live births, sex ratio, fetal body weights, or the occurrence of external, visceral, or skeletal malformations were observed in the offspring of rats and rabbits exposed to doses as high as 12.26 or 13.21 mg fluoride/kg/day, respectively, as sodium fluoride in drinking water consumed on gestational days 6–15 or 6–19, respectively (Heindel et al. 1996). Similarly, no developmental effects were observed in offspring of rats drinking water containing at least 11.2 mg fluoride/kg/day as sodium fluoride on gestational days 1–20 (Collins et al. 1995). An increase in the average number of fetuses per litter with at least three skeletal variations was seen at the highest dose tested (11.4 mg fluoride/kg/day); however, this was associated with decreased maternal water and food consumption and decreased body weight gain.

Bone morphology of weanling Sprague-Dawley rats from dams that received 21 mg fluoride/kg day for 10 weeks prior to breeding and during gestation was examined with both light and electron microscopy. No pathological changes were seen, suggesting that although fluoride is transported across the placenta, the amount transported was not sufficient to affect fetal bone development (Ream et al. 1983). There were no developmental effects of fluoride in the first litter of an extended two-litter reproduction study in UPj:TUC(SD)spf rats that were fed diets containing 23 or 2.8 mg fluoride/kg/day (two litters from each dam) (Marks et al. 1984). However, the second litters born to mothers in the high-fluoride group had a higher number of abnormal newborns and affected litters than were found in the low-fluoride group. The significance of this finding is unclear because the effect was not analyzed statistically.

Wild and domestic animals may be more sensitive than laboratory animals to developmental effects of fluoride. Stunted growth (Krook and Maylin 1979) and lameness (Maylin and Krook 1982) have been reported in calves that foraged on land downwind of an aluminum plant. Severe dental fluorosis confirmed high levels of fluoride ingestion. Mink kits that were born to mothers fed 9.1 mg fluoride/kg/day and fed the same feed after weaning exhibited a marked decrease in survivability (14% at 3 weeks, compared with 86% for the control) (Aulerich et al. 1987). There was no effect at the next lower dose. No further clinical details were provided for these pups. However, survival of the females exposed to that level was also decreased (17% at the end of the trial [382 days], compared with 100% for the control), so it is not clear if the kit effects were secondary to maternal toxicity. The only clinical signs in the adult mink were general unhealthiness, hyperexcitability, and lethargy a few days before they died. No lameness was observed.

3.2.2.7 Cancer

Numerous epidemiological studies have examined the issue of a connection between fluoridated water and cancer. The weight of evidence indicates that no such connection exists. However, all of the investigations were ecologic studies, and the sensitivity limit of even the most sensitive analysis in these studies appears to be a 10–20% increase. Since any carcinogenic effect of fluoride at the levels found in water supplies would probably be below this level of sensitivity, a National Toxicology Program (NTP) cancer bioassay was conducted to assess the effect of fluoride on cancer incidence in animals (Bucher et al. 1991; NTP 1990). The NTP study found equivocal evidence of a fluoride-related increase in osteosarcomas in male rats, and no evidence of any fluoride-related neoplasm in female rats or male or female mice. A study sponsored by Proctor and Gamble (Maurer et al. 1990) found no evidence of fluoride carcinogenicity in either male or female rats. Both studies contain limitations that preclude strong conclusions. The NTP is presently carrying out additional experiments on the relationship, if any, between fluoride and cancer. The International Agency for Research on Cancer (IARC) reviewed the literature on fluoride carcinogenicity in 1982. It concluded that there is no evidence from epidemio-

logical studies of an association between fluoride ingestion and human cancer mortality, and the available data are inadequate for an evaluation of the carcinogenicity of sodium fluoride in experimental animals (IARC 1982). Several major cancer bioassays of fluoride have been conducted since the IARC review.

Data suggesting that increased fluoride exposure from drinking water supplies is associated with an increase in cancer incidence come from the study published by Yiamouyiannis and Burk (1977) comparing the cancer incidence rates in 10 U.S. cities with artificial fluoridation and 10 cities without fluoridation. The authors of the study interpret these data as showing that cancer mortality was higher in the cities with artificially fluoridated water. Data from this study have been re-analyzed several times in an attempt to further explore the hypothesis that fluoridation of water supplies causes cancer (Chilvers 1982, 1983; Doll and Kinlen 1977; Hoover et al. 1976; Kinlen and Doll 1981; Oldham and Newell 1977; Taves 1977). None of these re-analyses provided evidence of a positive association between fluoridation of water supplies and cancer of any of the sites considered. The re-analyses attributed the positive association between fluoride exposure and cancer reported by Yiamouyiannis and Burk (1977) to dissimilarities in age, race, sex, and demographic factors for the populations studied. Other studies of large populations, both in the United States and Great Britain, have identified no relationship between artificially or naturally occurring fluoride in drinking water and an increase in cancer incidence (Griffith 1985; Hoover et al. 1991; Kinlen 1975). An inverse relationship between fluoride levels and cancer of the oral cavity and pharynx has been reported to occur in Norway in populations whose drinking water contained low levels of fluoride (0.05–0.5 mg/L) (Glattre and Wiese 1979). Although the authors offered no detailed mechanism for the apparent protective effect, and did not conduct a formal analysis of possible confounding factors, they did present data indicating that biases due to tobacco consumption, rural and urban differences, and differences in the population sizes of the examined communities could not be the cause of the reduced cancer rates.

A recent epidemiological study (Hoover et al. 1991) examined >2,300,000 cancer deaths and >125,000 cancer cases in U.S. counties exposed to artificially fluoridated drinking water for up to 35 years. Taking into account the results of the NTP study described below, detailed analyses were conducted of cancers of the joints and bones (especially osteosarcomas), and cancers of the oral cavity and pharynx. The statistical evaluation was based on analysis of time trends in the observed/expected (O/E) ratios relative to duration of fluoridation. While elevated O/Es were observed for osteosarcomas in males, the O/E ratio was inversely related to duration of fluoridation. Thorough analyses of incidences of oral cancers and cancers at a variety of other sites were conducted by means of very sensitive statistical tests that were designed to detect changes as small as 10–20%. No consistent correlation between cancer incidence or mortality and duration of fluoridation was found. An addendum to the report noted that the age-adjusted national incidence of osteosarcoma increased by 18% in males for the years 1973–1980 and 1981–1987; most of the increase was due to a 53% increase in males under 20 years of age, and there was

a larger increase in fluoridated than nonfluoridated areas. A similar time-trend analysis to that done in the main report found no correlation between the cancer incidence O/E ratio and duration of fluoridation. Additional analyses also failed to find a relationship between osteosarcoma incidence in males and exposure to fluoridated water.

In general, occupational fluoride exposures result in much higher intake rates than does ingestion of artificially fluoridated drinking water. Actual absorbed doses from occupational exposure are not available, but urinary fluoride levels can reach 5.68 mg/L (Dinman et al. 1976c), compared with normal levels of about 1 mg/L (Spencer et al. 1970). Studies regarding cancer from occupational inhalational exposure to hydrofluoric acid fumes and dust from cryolite were discussed in Section 3.2.1.7.

The NTP conducted two chronic oral bioassays of fluoride administered as sodium fluoride (0, 25, 100, or 175 ppm) in drinking water, using F344/N rats and B6C3F$_1$ mice (Bucher et al. 1991; NTP 1990). The first study was considered compromised for reasons that will be discussed below. However, pathology data from the first study were used in determining the doses for the second study. The diet used in the second study was specially formulated to be low in fluoride, and contained 8.6 ppm fluoride; daily fluoride amounts administered in the food for control and experimental groups was 0.43 mg/kg/day in rats and 1.1 mg/kg/day in mice. Based on the total amount of fluoride ingested and the amount in the feces, and apparently assuming that none of the fluoride found in the feces was absorbed, Bucher et al. (1991) calculated that the average bioavailability of fluoride in the food over the course of the experiment was 60%. Assuming complete absorption of fluoride in the water, they estimated total fluoride intake (including fluoride in both water and diet) of control, low-, medium-, and high-dose male rats as 0.2, 0.8, 2.5, and 4.1 mg/kg/day, respectively. Similarly, the high doses for female rats, male mice, and female mice were 4.5, 8.1, and 9.1 mg/kg/day, respectively.

The study found osteosarcomas in the bone of 1/50 male rats in the mid-dose group and 3/80 of the high-dose male rats. An additional high-dose male had an extraskeletal osteosarcoma in subcutaneous tissue. Examination of radiographs did not reveal a primary site in bone for the extraskeletal tumor, suggesting that it was a soft-tissue tumor that later ossified. No osteosarcomas were found in the low-dose or control rats. One of the osteosarcomas in the high-dose group was missed on radiographic examination and in the necropsy, and found only on microscopic examination. Three of the tumors were in the vertebra and only one was in a long bone. This is unusual, as Bucher et al. (1991) stated that chemically-induced osteosarcomas usually appear in the long bones, rather than in the vertebrae. Statistical analysis found a significant dose-response trend in the four osteosarcomas of the bone ($p=0.027$), but no significant difference ($p=0.099$) in a pairwise comparison of the controls with the high-dose group. The probability value for the trend test was decreased ($p=0.010$) when the extraskeletal osteosarcoma was included, but the pairwise test was still not significant ($p=0.057$). Osteosarcomas are rarely observed in control male

rats in NTP studies; the historical incidence is 0.6% (range 0–6%). The rate in the high-dose group in this study was 3.75 or 5%, depending on whether or not the extraskeletal tumor is included. Tumor rates could not be compared with the historical controls because the diet generally used for NTP studies contains >20 ppm fluoride. Assuming the same bioavailability of 60%, the study report states that this would place the historical controls between the low- and mid-dose groups in the fluoride study. Conversely, the more extensive bone examinations used in the fluoride study, both at the macroscopic level and histologically, could have led to higher bone tumor levels being observed than in historical controls.

The average fluoride level in the bones of male rats in the high-dose group was 5,260 ppm. While similar bone fluoride levels were found in the bones of female rats and male and female mice, there was no evidence of treatment-related osteosarcomas in these groups. Osteosclerosis was observed in high-dose female rats, suggesting a stimulatory or mitogenic effect on osteoblasts (Marie and Hott 1986). Osteosclerosis was not observed in mice, despite the higher dose. Osteosarcomas were observed in one low-dose male mouse, one low-dose female mouse, and one control female mouse. There was also one osteoma in a control female mouse. No osteosarcomas were observed at mid- or high-dose levels in female rats or male or female mice. The study authors stated that the absence of treatment-related osteosarcomas in female rats and male and female mice may have limited relevance to the findings in male rats. Results in the literature are mixed as to whether there is a sex-linked response in bone tumor formation (Litvinov and Soloviev 1973; NCI 1978).

Increased tumor incidence in rats or mice was noted in a few other tissues, but was not considered biologically significant. For example, the combined incidence of squamous cell papillomas and carcinomas in the oral mucosa was marginally increased in the high-dose male and female rats and thyroid follicular cell neoplasms were marginally increased in the high-dose male rats. Neither increase was statistically significant, and both types of neoplasms lacked a supporting pattern of increased preneoplastic lesions. Similarly, increased levels of keratoacanthomas were observed in high-dose female rats, but were not considered biologically significant because other benign neoplasms arising from stratified squamous epithelium was found in the controls. Malignant lymphoma and histiocytic sarcoma incidence in female high-dose mice was marginally increased (combined rate 30%), but the increase was not considered biologically significant. The incidence was well within the range of historical controls at the study laboratory (18–48%) and at all NTP laboratories (10–74%). The incidence of hepatocellular neoplasms in male and female mice of the treatment and control groups was higher than in historical controls. The study authors noted similar increases in other NTP studies that were conducted contemporaneously, and suggested that they may be associated with increased animal weight. Hepato-cholangiocarcinomas, which are rare liver neoplasms, were identified in the original pathology examination in five treated male mice, four treated female mice, and one control female mouse. The

Pathology Working Group reclassified all of the neoplasms (except one in a high-dose female mouse and one in a control female mouse) as hepatoblastomas, because they contained well-defined populations of cells that resembled embryonal liver cells more closely than they did biliary cells. The dose levels at which the reclassified hepatocholangiocarcinomas were found were not reported.

Interpretation of this study is further complicated because higher doses might have been tolerated in both the rat and the mouse studies (NTP 1990). Fluoride-related tooth abnormalities found in the study included dental attrition in males of both species that was dose-related in rats but not in mice, dentine dysplasia in both genders of both species, and tooth deformities in male rats. No other treatment-related toxic effects were found in any group, and there was no evidence of decreased body weight gain in any group. Higher fluoride levels may have affected the teeth of the male rats so severely as to interfere with the animals' ability to eat. However, it appears that the mice and possibly the female rats could have tolerated a higher dose.

Based on the finding of a rare tumor in a tissue known to accumulate fluoride, but not at the usual site for chemically-associated osteosarcomas, a weakly significant dose-related trend, and the lack of supporting data in female rats and mice of either gender, the NTP concluded that there was "equivocal evidence of carcinogenic activity of sodium fluoride in male F344/N rats." NTP defined equivocal evidence of carcinogenic activity to be a situation where the results show "a marginal increase in neoplasms that may be chemically related." NTP further concluded that there was no evidence that fluoride was carcinogenic at doses up to 4.73 mg/kg/day in female F344/N rats, or at doses up to 17.8 and 19.9 mg/kg/day in male and female B6C3F_1 mice, respectively.

The first chronic study in this series conducted by NTP was a 2-year cancer study in B6C3F_1 mice and F344/N rats using a semisynthetic diet containing 2.1 ppm fluoride and fluoride provided in drinking water as sodium fluoride at 0, 10, 30, or 100 ppm. Several nontreatment-related clinical signs developed in rats, including corneal lesions and head tilt. Analysis of the diet revealed marginal to marked deficiencies in manganese, chromium, choline, and vitamins B_{12} and D. Based on these findings, the study was considered compromised, but the results were used to aid in dose selection for the second study. Only the following unverified pathology findings were reported: (1) one osteosarcoma in the occipital bone of one low-dose male rat; (2) one osteoma in the vertebra of a male control mouse; (3) one subcutaneous osteosarcoma in one female high-dose mouse; and (4) no osteosarcomas were found in female rats (male mice were not mentioned).

A study sponsored by Proctor and Gamble examined the carcinogenic potential of sodium fluoride administered in feed to Sprague-Dawley rats (Maurer et al. 1990). One group of controls was fed laboratory chow, and another control group was fed a semisynthetic low-fluoride diet. The control group

fed the low-fluoride diet received 0.14 (males) or 0.18 (females) mg fluoride/kg/day as sodium fluoride. The fluoride level in the laboratory chow was not determined. Treatment groups ingested 1.8, 4.5, or 11.3 mg fluoride/kg/day in the diet as sodium fluoride. Fluoride bioavailability was not determined and water fluoride levels were not reported. However, the high dose (11.4 mg fluoride/kg/day) was probably close to the maximum tolerated dose (MTD), since there was a 30% decrease in body weight gains of both sexes. The study was terminated early because of high mortality in all treatment and control groups. The cause of the elevated level of mortality was not determined. Fluoride-related toxicity was observed in the teeth, stomach, and bones.

Evidence of fluoride toxicity in the Maurer et al. study included dose-related hyperostoses in males and females, tooth abnormalities, and stomach inflammation. Fluoride levels in the bone ash of the high-dose males and females were 16,761 and 14,438 ppm, respectively. Primary tumors in target tissues as reported by the study authors were one fibroblastic sarcoma with areas of osteoid formation in a high-dose male, one osteosarcoma in a low-dose female, one chordoma in a mid-dose male, one chondroma each in a mid-dose male and a low-dose female, one odontoma in a laboratory-chow control, and one stomach papilloma in a low-fluoride control. Re-examination of tissue slides as part of a review of the study by the Carcinogenicity Assessment Committee, Center for Drug Evaluation and Research, Food and Drug Administration (CAC/CDER/FDA) revealed an additional osteosarcoma in a low-dose female and one osteosarcoma in a high-dose male. Statistical analysis of the incidence of bone tumors found no dose-response relationship (CDER 1991).

Several limitations of the study were not apparent in the study report, but were noted in the CAC review (CDER 1991). The low-fluoride diet may not have allowed normal growth and development, since pale livers and gastric hairballs were observed in all study animals except those fed laboratory chow. The diet and water were often above specifications for minerals, ions, and vitamins. A virus was found during the pretest period and its continued presence during the study was suspected; this may have compromised the health of the animals. The finding of bone tumors missed by the contract laboratory raised questions about the adequacy of the examination at gross necropsy. Finally, bone sections from only 50–80% of the mid- and low-dose animals were analyzed microscopically. The CAC review concluded that there are "flaws and uncertainties in the studies that keep them from providing strongly reassuring data." However, the committee concluded that the study results reaffirm the negative finding of the NTP study in female rats, and do not reinforce the equivocal finding in male rats.

3.2.3 Dermal Exposure

Several human and animal studies investigating the health effects following accidental dermal exposure to hydrofluoric acid were located. In addition, many of the human and animal studies investigating the health effects of inhalation exposure to fluorine or hydrogen fluoride found dermal/ocular effects due to the irritating effects of these chemicals. (In this section, hydrogen fluoride refers to the gas while hydrofluoric acid refers to the liquid.) One study regarding dermal exposure to sodium fluoride was located. Fluorine causes severe irritation of the eyes and skin and can severely burn the skin at high concentrations. Hydrofluoric acid is a caustic acid and can produce severe tissue damage either as the water solution, or in the anhydrous form (hydrogen fluoride). Hydrofluoric acid can also rapidly penetrate the skin and cause systemic effects, especially cardiac arrhythmias. If left untreated, death can result.

3.2.3.1 Death

Hydrofluoric Acid. Fatalities from dermal fluoride exposure occur most frequently from accidental exposure to hydrofluoric acid in an occupational setting. The actual systemic doses are seldom known. However, the extent and severity of the burns, and occasionally, clinical chemistry values are reported. Death following hydrofluoric acid burns to the extremities, in the absence of inhalation exposure, is due to cardiac arrhythmias, with pronounced hypocalcemia, hyperkalemia, and hypomagnesemia. Ion pump disruption is thought to be the mechanism of systemic toxicity. Hydrofluoric acid exposure of the face has also resulted in death due to respiratory insufficiency, but the respiratory effects are likely to be due to concurrent inhalation exposure. Depending on the extent of the body surface exposed and the effectiveness of medical treatment, death usually occurs within a few hours (Chan et al. 1987; Chela et al. 1989; Kleinfeld 1965).

A patient with hydrofluoric acid burns on his leg involving 8% of his body surface area died from intractable cardiac arrhythmia, presumably secondary to the depletion of ionized calcium by the fluoride ion (Mullett et al. 1987). Serum fluoride level 4 hours after the burn injury was reported to be 9.42 µg/mL, about 400 times the value reported as normal for that age and sex. A 23-year-old man who sustained second and third degree burns of his thighs, covering 9–10% of his body surface area died of cardiac arrhythmia 17 hours after exposure (Mayer and Gross 1985); serum fluoride was 4.17 µg/mL.

The death of a chemist who sustained first- and second-degree burns of the face, hands, and arms when a vat containing hydrofluoric acid accidentally broke has been reported (Kleinfeld 1965). This 29-year-old male died 10 hours after admission to the hospital. Postmortem examination showed severe tracheobronchitis and hemorrhagic pulmonary edema. A petroleum refinery worker was splashed in the face with 100% anhydrous hydrofluoric acid (Tepperman 1980). The burn produced acute systemic

fluoride poisoning with profound hypocalcemia and hypomagnesemia. The patient died <24 hours after exposure. A young woman splashed in the face with hydrofluoric acid died a few hours after exposure occurred (Chela et al. 1989). The autopsy revealed severe burns of the skin and lungs, with pulmonary hemorrhagic edema produced by hydrofluoric acid and its vapor.

No studies were located regarding lethality in humans after dermal exposure to fluorine or fluoride, and no studies were located regarding lethality in animals after dermal exposure to fluorine, hydrofluoric acid, or fluoride.

3.2.3.2 Systemic Effects

No studies were located regarding gastrointestinal, hematological, musculoskeletal, endocrine, or body weight effects in humans or animals after dermal exposure to fluorine, hydrofluoric acid, or fluoride.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category of exposure to fluorine are recorded in Table 3-4. The highest NOAEL and all reliable LOAEL values for systemic effects in each species and duration category of exposure to hydrogen fluoride or hydrofluoric acid are recorded in Table 3-5. All reliable LOAEL values for systemic effects in each species and duration category for fluoride are recorded in Table 3-6.

Respiratory Effects.

Hydrofluoric Acid. Respiratory effects including pulmonary edema, tracheobronchitis, and pulmonary hemorrhagic edema have been reported in humans following acute dermal exposure of the face to hydrofluoric acid (Chela et al. 1989; Kleinfeld 1965). However, the pulmonary effects are likely to be due to concomitant inhalation of the acid vapor. As two of these cases were occupational accidents and the third was a homicide, no doses could be estimated from the information provided.

No studies were located regarding respiratory effects in humans after dermal exposure to fluorine or fluoride, and no studies were located regarding respiratory effects in animals after dermal exposure to fluorine, hydrofluoric acid, or fluoride.

Cardiovascular Effects. Cardiac arrhythmias are found following acute dermal exposure to hydrofluoric acid in humans (Mayer and Gross 1985; Mullett et al. 1987). A man who received a hydrofluoric acid burn on the arm covering 5% of the body experienced repeated ventricular fibrillation episodes, but survived following administration of intravenous calcium chloride, subcutaneous calcium gluconate, and excision of the burn area (Buckingham 1988). These cardiovascular effects are believed to result from the strong binding of fluoride to calcium, which produces hypocalcemia. Serum calcium is

critical for proper ion transport in neuromuscular synapses; hypocalcemia can cause the ventricles not to contract properly.

No studies were located regarding cardiovascular effects in humans after dermal exposure to fluorine or fluoride, and no studies were located regarding cardiovascular effects in animals after dermal exposure to fluorine, hydrofluoric acid, or fluoride.

Hepatic Effects.

Hydrofluoric Acid. Elevated SGOT, serum glutamic pyruvic transaminase (SGPT), and lactate dehydrogenase levels were found in a man who was splashed in the face and on the neck with a mixture of 10% hydrofluoric acid and sulfuric acid (Braun et al. 1984). The elevated SGOT and SGPT levels were attributed to either muscle necrosis or temporary liver damage caused by toxic metabolic products from necrotic tissue.

Renal Effects.

Hydrofluoric Acid. A 49-year-old man who was splashed in the face and on the neck with a mixture of hydrofluoric acid and sulfuric acid became oliguric for a brief period on the day after the accident, and then became anuric (Braun et al. 1984). Concomitant inhalation exposure is likely, and the effect of the sulfuric acid is unknown.

Dermal Effects.

Fluorine. When the shaved backs of New Zealand rabbits were exposed to fluorine gas under 40 pounds of pressure for 0.2–0.6 seconds at distances of 0.5–1.5 inches, the resulting burn appeared to be thermal, rather than chemical in nature (Stokinger 1949). Exposure for 0.2 seconds produced an ischemic area about ¼ inch in diameter, surrounded by an erythematous area. This became a superficial eschar that sloughed off within 4 days, revealing normal epidermis. The longer exposures produced a flash of flame that resulted in combustion of hair, singeing, and erythema over an area several times the area of the primary burn. Coagulation necrosis and charring of the epidermis was also reported. The wound healed within 13 days. The burns resembled those produced by an oxyacetylene flame, rather than those made by hydrofluoric acid, and so were characterized as thermal, rather than chemical. However, it is not clear if the difference from the hydrofluoric acid burn is due to the shorter exposure to fluorine.

Hydrogen Fluoride/Hydrofluoric Acid. Dermal exposure to hydrogen fluoride can cause irritation of the skin and mucous membranes. Residents exposed to hydrogen fluoride following an accidental release reported a number of skin effects including itching, burning, and rash; 43.8% of the highly exposed

residents reported severe skin problems, as compared to 5.3% of nonexposed residents (Dayal et al. 1992). Two years after the accident, severe skin problems were reported by 21.9% of the high exposure group compared to 2.7% of the control group. Severe dermal effects have not been reported from dermal exposure to hydrogen fluoride gas, but it is not clear if this is because the gas does not cause such effects, or because concentrations high enough to cause severe effects were not tested. Dermal exposure to hydrofluoric acid results in extensive skin burns (Chela et al. 1989). Hydrofluoric acid quickly penetrates into soft tissues and causes necrosis. As a result of cell membrane destruction, the fluoride ion has easy access to lymph and the venules, can be distributed rapidly, and can cause significant adverse effects such as inhibition of glycolytic enzymes, hypocalcemia, and hypomagnesia. Untreated burns of the fingers can result in loss of fingers.

“Smarting" of exposed skin occurred in humans within 1 minute of exposure to hydrogen fluoride at about 122 ppm fluoride (Machle et al. 1934). This was the highest concentration that two male volunteers could tolerate for >1 minute. Repeated exposures did not reveal any habituation.

There are many reports of hydrofluoric acid skin burns in humans. In one case, a 23-year-old man received fatal second- and third-degree burns over 9–10% of his body from a 70% hydrofluoric acid spill (Mayer and Gross 1985). The patient died 17 hours after exposure due to cardiac arrhythmias. Two case studies of accidental dermal exposure of the hands to hydrofluoric acid (5–7%) reported serious dermal injury following exposures from 45 minutes to 6 hours (Roberts and Merigian 1989). Topical treatment with calcium gluconate prevented loss of nails. Other case reports are discussed in Section 3.2.3.1.

Exposure to hydrogen fluoride levels approaching the LC_{50} can cause lesions of the face in rats (Haskell Laboratory 1988). Rats exposed to hydrogen fluoride (whole body) at a concentration of approximately 1,395 ppm fluoride for 60 minutes were observed to have erythema of an unspecified severity of the exposed skin (Wohlslagel et al. 1976).

Subcutaneous hemorrhages around the eyes and on the feet developed in rats exposed to 8.2 or 31 ppm fluoride as hydrogen fluoride for 6 hours/day, 6 days/week for 5 weeks (Stokinger 1949). The effect was more severe at the higher exposure level. Dogs exposed to 31 ppm fluoride for the same time periods developed inflammation of the scrotal epithelium.

The concentration of hydrofluoric acid and the length of exposure affect the severity of dermal lesions (Derelanko et al. 1985). Rabbits exposed to a hydrofluoric acid solution of 0.01% for 5 minutes had visible skin lesions, whereas exposure to 2% hydrofluoric acid for 1 minute did not produce lesions. A longer exposure of 1–4 hours to 2% hydrofluoric acid solution produced necrotic lesions on the backs of rabbits (Derelanko et al. 1985).

The application of 0.2 mL of a 47% hydrofluoric acid solution to the shaved backs of New Zealand rabbits over a surface of 1¼ inches produced no immediate reaction (Stokinger 1949). The material was held in place by lanolin and allowed to dry for 24 hours. Within a few days of exposure, erythema and dark spots of liquefaction necrosis appeared. Multiple eschars were formed in the necrotic areas. These wounds healed more slowly than those produced by fluorine gas. Healing did not near completion until 27 days after exposure.

Fluoride. Sodium fluoride applied topically to the abraded skin of Sprague-Dawley rats (0.5 or 1.0%) for 24 hours produced both morphological and biochemical changes (Essman et al. 1981). At 0.5%, the abraded surface showed focal superficial necrosis of the epidermis. At 1.0%, the abraded surface showed edema and vacuolization. There was marked edema of the dermis with inflammation. Skin histamine concentrations were also increased following application of 0.5 or 1% sodium fluoride to shaved-only or epidermally abraded skin, although the variance of these measurements was quite high.

The highest NOAEL values and all reliable LOAEL values for dermal effects of fluorine exposure for each species and duration category are recorded in Table 3-4. The highest NOAEL and all reliable LOAEL values for dermal effects of hydrogen fluoride exposure for each species and duration category are recorded in Table 3-5. All reliable LOAEL values for dermal effects of fluoride exposure are recorded in Table 3-6.

Ocular Effects.

Fluorine. Volunteers (19–50 years of age) were exposed to 10 ppm fluorine for 15 minutes without discomfort or irritation of the eyes or nose (Keplinger and Suissa 1968). However, repeated exposures to 23 ppm fluorine for 3–5 minutes every 15 minutes over a 2–3-hour period caused slight eye irritation. Exposure was through a face mask that covered the eyes and nose but not the mouth. Eye irritation was also reported following exposure to 50 ppm for 3 minutes and 67 and 78 ppm for 1 minute. Exposure to 100 ppm was very irritating and became uncomfortable after a few seconds. At this concentration, the subjects reported that the eyes burned and felt as though they were covered by a film.

Eye irritation, evidence by pawing of eyes, was observed in rats exposed to 140 or 175 ppm fluorine for 30 or 5 minutes, respectively, and in dogs exposed to 68 or 93 ppm fluorine for 60 or 15 minutes, respectively. In experiments with exposure for durations of 15–60 minutes, eye and nose irritation was reported only at ~50% of the LC_{50}. Similar results were obtained with Swiss-Webster mice, New England guinea pigs, and New Zealand rabbits.

Hydrogen Fluoride/Hydrofluoric Acid. Marked conjunctival irritation in humans within 1 minute of exposure to hydrogen fluoride at about 95 mg fluoride/m^3 (Machle et al. 1934). This was the highest concentration that two male volunteers could tolerate for >1 minute. At 48 mg fluoride/m^3, conjunctival and nasal irritation were still marked, and tickling and discomfort of the nasal passages were reported. A

Table 3-4. Levels of Significant Exposure to Fluorine - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
ACUTE EXPOSURE						
Systemic						
Human	1d 1min/d	Dermal	67	78 (skin irritation)		Keplinger and Suissa 1968 fluorine
		Ocular		67 (eye irritation)		
Human	1d 3min/d	Ocular	10	50 (eye irritation)		Keplinger and Suissa 1968 fluorine
Human	1d 5min/d	Ocular	10			Keplinger and Suissa 1968 fluorine
Human	1d 3-5min every 15 min for 2-3 hr	Dermal		23 (slight skin irritation)		Keplinger and Suissa 1968 fluorine
		Ocular		23 (slight eye irritation)		
Human	1d 0.5min/d	Ocular		100 (eye irritation)		Keplinger and Suissa 1968 fluorine
Rat (Osborne-Mendel)	1d 60min/d	Ocular	93			Keplinger and Suissa 1968 fluorine
Rat (Osborne-Mendel)	1d 5min/d	Ocular	88	175 (eye irritation)		Keplinger and Suissa 1968 fluorine
Rat (Osborne-Mendel)	1d 15min/d	Ocular	195			Keplinger and Suissa 1968 fluorine

Table 3-4. Levels of Significant Exposure to Fluorine - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
Rat (Osborne-Mendel)	1d 30min/d	Ocular	70	140 (eye irritation)		Keplinger and Suissa 1968 fluorine
Mouse (Swiss-Webster)	1d 5min/d	Ocular	300	467 (eye irritation)		Keplinger and Suissa 1968 fluorine
Mouse (Swiss-Webster)	1d 15min/d	Ocular	188			Keplinger and Suissa 1968 fluorine
Mouse (Swiss-Webster)	1d 30min/d	Ocular	113			Keplinger and Suissa 1968 fluorine
Dog (NS)	1d 15min/d	Ocular	39	93 (eye irritation)		Keplinger and Suissa 1968 fluorine
Dog (NS)	1d 60min/d	Ocular	38	68 (eye irritation)		Keplinger and Suissa 1968 fluorine

d = day(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; ppm = parts per million.

Table 3-5. Levels of Significant Exposure to Hydrogen Fluoride - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
ACUTE EXPOSURE								
Systemic								
Rat	1 d 60min/d	Ocular	98	120	(lacrimation)			Rosenholtz et al. 1963 hydrogen fluoride
Rat	1 d 15min/d	Ocular	292	357	(lacrimation)			Rosenholtz et al. 1963 hydrogen fluoride
Rabbit	1 d 1-4hr/d	Dermal	2% per min			2% per hr	(necrotic lesions)	Derelanko et al. 1985 hydrogen fluoride
INTERMEDIATE EXPOSURE								
Systemic								
Human	15-50 d 6 hr/d	Dermal		2.98	(stinging sensation on skin)			Largent 1960 hydrogen fluoride
		Ocular		2.98	(stinging sensation in eyes)			
Rat (NS)	5 wks 6d/wk 6hr/d	Dermal		8.2	(subcutaneous hemorrhage around the eyes and on the feet)			Stokinger 1949 hydrogen fluoride
Mouse (NS)	5 wks 6d/wk 6hr/d	Dermal		31	(subcutaneous hemorrhage around the eyes and on the feet)			Stokinger 1949 hydrogen fluoride

Table 3-5. Levels of Significant Exposure to Hydrogen Fluoride - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
Reproductive						
Dog (NS)	5 wks 6d/wk 6hr/d		8.2	31 (ulceration of the scrotum)		Stokinger 1949 hydrogen fluoride

d = day(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; wk = week(s); ppm = parts per million

Table 3-6. Levels of Significant Exposure to Fluoride - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL				Reference Chemical Form
				Less Serious		Serious		
Rat (Sprague-Dawley)	1 d 24hr/d	Dermal		0.5%	(superficial necrosis, moderate edema, PMN infiltration)	1%	(extensive necrosis, marked edema, degenerating mast cells)	Essman et al. 1981 sodium fluoride

d = day(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PMN = polymorphnuclear leukocyte

concentration of 24.7 mg fluoride/m^3 produced mild irritation of the nose and eyes and irritation of the larger air passages. This concentration could be tolerated for "several" minutes (at least 3 minutes). The authors of this study reported some difficulties with their measurements of exposure. Repeated exposures did not reveal any habituation. Mild eye irritation was observed in five volunteers exposed 6 hours/day for 10 days, to hydrogen fluoride at concentrations averaging from approximately 2–4 mg/m^3 (Largent 1960). This study is limited by the inadequacy of both the experimental details and the description of effects observed.

Severe symptoms of eye problems were reported by 63.2% of Texas residents exposed to high levels of hydrogen fluoride following an accidental release (Dayal et al. 1992). The most commonly reported eye effects were redness, itching, and burning or irritation. Two years after the accident, 11.5% of the population still reported severe eye problems. In nonexposed residents, the prevalence of severe symptoms within the first month of the accident was 7.4; 2 years later, the prevalence was 4.9.

Some evidence of delayed ocular damage due to persistence of the fluoride ion was observed 4 days after a 3-year-old girl accidentally sprayed a hydrofluoric-acid-containing product in her eyes (Hatai et al. 1986). Opacification of the corneal epithelium and thrombosis of the conjunctival vessels were seen. These changes were not permanent; after 30 days, the eyes returned to normal, and vision was 20/20. However, it is difficult to generalize from this report as the product contained both hydrofluoric acid and phosphoric acid at unspecified concentrations.

Hydrogen fluoride levels approaching the LC_{50} can cause corneal opacity in rats (Haskell Laboratory 1988), while slight ocular irritation was observed in rats exposed to levels as low as 6% of the LC_{50} (Rosenholtz et al. 1963).

McCulley et al. (1983) concluded that the greater severity of hydrofluoric acid eye injuries compared to injuries from other inorganic acids at comparable strengths probably results from the destruction of the corneal epithelium allowing substantial penetration of the fluoride ion into the corneal stroma and underlying structures.

The highest NOAEL values and all reliable LOAEL values for ocular effects of fluorine exposure for each species and duration category are recorded in Table 3-4. The highest NOAEL and all reliable LOAEL values for ocular effects of hydrogen fluoride exposure for each species and duration category are recorded in Table 3-5.

No studies were located regarding the following effects in humans and animals after dermal exposure to fluorine, hydrofluoric acid, or fluoride:

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

In general, positive genotoxicity findings occurred at doses that are highly toxic to cells and whole animals. Lower doses were generally negative for genotoxicity. Tables 3-7 and 3-8 present the results of more recent assays.

The *in vivo* genotoxicity of fluoride has been tested in humans and animals following inhalation, oral, or parenteral exposure. No alterations in the occurrence of sister chromatid exchange were observed in a population living in areas with high levels of fluoride (4.8 ppm) in the drinking water (Li et al. 1995b). Mixed results have been reported in animal studies examining the clastogenic potential of hydrogen fluoride and sodium fluoride. Increases in the occurrence of chromosome aberrations were found in the bone marrow cells of rats exposed by inhalation to 1.0 mg/m^3 hydrogen fluoride 6 hours/day, 6 days/week for 1 month (Voroshilin et al. 1975) and in mouse bone marrow cells following oral, intraperitoneal, or subcutaneous exposure to sodium fluoride (Pati and Bunya 1987). However, other studies did not find significant alterations in the occurrence of chromosome aberrations in mouse bone marrow cells following oral exposure (Kram et al. 1978; Martin et al. 1979). Additionally, no alterations in sister chromatid exchange occurrence were observed in mouse or Chinese hamster bone marrow cells following oral exposure (Kram et al. 1978; Li et al. 1987b). Intraperitoneal injection of sodium fluoride resulted in an increase in micronuclei in mouse bone marrow cells (Pati and Bhunya 1987); no alterations were observed in rat bone marrow cells following oral exposure (Albanese 1987). Hydrogen fluoride was negative for dominant lethal mutations following inhalation exposure to hydrogen fluoride in C57B1 mice (Voroshilin et al. 1975). A study in *Drosophila melanogaster* in which reproductive parameters were measured as an indicator of genotoxicity, significant reductions in the number of eggs per female and male fertility were observed following inhalation exposure to hydrogen fluoride (Gerdes et al. 1971b). The maximum lethality to adults of one of the two tested strains was 60%; under most of the test conditions, the lethality was #40%.

Table 3-7. Genotoxicity of Fluoride *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Form
Prokaryotic organisms:					
Salmonella typhimurium	Gene mutation	–	–	Martin et al. 1979; NTP 1990; Tong et al. 1988	NaF
Eukaryotic organisms:					
Human lymphocytes	Chromosomal aberrations	No data	+	Albanese 1987	NaF
Human lymphocytes	Chromosomal aberrations	No data	–	Thomson et al. 1985	NaF, KF
Human fibroblasts	Chromosome aberrations	No data	+	Tsutsui et al. 1984c	NaF
Human fibroblasts	Chromosomal aberrations	No data	–	Tsutsui et al. 1995	NaF
Human diploid IMR-90 cells	Chromosomal aberrations	No data	+	Oguro et al. 1995	NaF
Human lymphocytes	Sister chromatid exchange	No data	–	Thomson et al. 1985; Tong et al. 1988	NaF
Human lymphocytes	Sister chromatid exchange	No data	–	Thomson et al. 1985	KF
Human lymphoblasts	Gene mutation	+	+	Caspary et al. 1988	NaF
Human fibroblasts	Unscheduled DNA synthesis	No data	+	Tsutsui et al. 1984c	NaF
Syrian hamster embryo cell	Chromosomal aberrations	No data	+	Tsutsui et al. 1984b	NaF
Syrian hamster embryo cell	Sister chromatid exchange	No data	+	Tsutsui et al. 1984b	NaF
Syrian hamster embryo cell	Unscheduled DNA synthesis	No data	+	Tsutsui et al. 1984b	NaF
Chinese hamster ovary cells	Sister chromatid exchange	No data	–	Li et al. 1987b	NaF
Chinese hamster ovary cells	Sister chromatid exchange	No data	–	Tong et al. 1988	NaF
Chinese hamster ovary cells	Sister chromatid exchange	+	+	NTP 1990	NaF
Chinese hamster ovary cells	Chromosomal aberrations	+	+	Aardema et al. 1989	NaF
Chinese hamster ovary cells	Chromosomal aberrations	–	+	NTP 1990	NaF
Chinese hamster V79 cells	Gene mutation	No data	–	SlameÁová et al. 1992	NaF
Mouse lymphoma cells	Gene mutation	No data	(+)	Cole et al. 1986	NaF

Table 3-7. Genotoxicity of Fluoride *In Vitro (continued)*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Form
Mouse lymphoma cells	Gene mutation	+	+	Caspary et al. 1987, 1988; NTP 1990	NaF
Mouse lymphoma cells	Gene mutation	+	+	Caspary et al. 1987	KF
Rat hepatocytes	DNA repair	No data	–	Tong et al. 1988	NaF
Rat liver epithelium cells	Gene mutation	No data	–	Tong et al. 1988	NaF
Rat vertebral body derived cells	Chromosome aberrations	No data	+	Mihashi and Tsutsui 1996	NaF
Rat bone marrow cells	Chromosome aberrations	No data	(+)	Khalil 1995	NaF, KF
Rat bone marrow cells	Sister chromatid exchange	No data	–	Khalil and Da'dara 1994	NaF, KF

- = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid; KF = potassium fluoride; NaF = sodium fluoride

Table 3-8. Genotoxicity of Fluoride *In Vivo*

Species (test system)	End point	Results	Reference	Form
Human lymphocytes (oral exposure)	Sister chromatid exchange	–	Li et al. 1995b	NR
Rat bone marrow cells (oral exposure)	Micronuclei	–	Albanese 1987	NaF
Rat bone marrow	Chromosome aberrations	+	Voroshilin et al. 1975	HF
Rat testis cells (oral exposure)	DNA strand breaks	–	Skare et al. 1986	NaF
Mouse (C57B1)	Dominant lethal	–	Voroshilin et al. 1975	HF
Mouse (Harlan Sprague-Dawley)	Sperm head abnormality	–	Li et al. 1987a	NaF
Mouse bone marrow and testis cells (oral exposure)	Chromosome aberrations	–	Martin et al. 1979	NaF
Mouse bone marrow cells (oral, intraperitoneal, or subcutaneous exposure)	Chromosome aberrations	+	Pati and Bhunya 1987	NaF
Mouse bone marrow cells (intraperitoneal exposure)	Micronuclei	+	Pati and Bhunya 1987	NaF
Mouse bone marrow cells (oral exposure)	Chromosome aberrations	–	Kram et al. 1978	NaF
Mouse bone marrow cells (oral exposure)	Sister chromatid exchange	–	Kram et al. 1978	NaF
Chinese hamster bone marrow cells (oral exposure)	Sister chromatid exchange	–	Li et al. 1987b	NaF

– = negative result; + = positive result; DNA = deoxyribonucleic acid; HF = hydrogen fluoride; NaF = sodium fluoride; NR = not reported

3.4 TOXICOKINETICS

The majority of data on the toxicokinetics of fluoride focus on sodium fluoride and hydrofluoric acid. Data regarding the toxicokinetics of calcium fluoride and other fluorides in human or animals are limited. While radioactive isotopes are useful in toxicokinetic studies, this use is limited in studies of fluoride because the fluorine isotope ^{18}F has a short half-life (Wallace-Durbin 1954). Only one animal study and no human studies were located regarding the toxicokinetics of fluorine.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Data providing information on absorption rates exist on the inhalation exposure of humans to mixtures of hydrogen fluoride and fluoride dusts, and inhalation exposure of animals to hydrogen fluoride. Animal data also exist showing that fluorine is absorbed.

Fluorine. No data were located regarding the absorption of fluorine in humans. Hepatic and renal effects were observed in mice following exposure to fluorine for periods up to 60 minutes (Keplinger and Suissa 1968). This indicates that the fluoride ion was systemically available following the exposure. Fluoride, rather than fluorine, is the agent that is toxicologically active systemically, since fluorine is too reactive to be absorbed unchanged. Similarly, the finding of elevated fluoride levels in bones, teeth, and urine during intermediate-duration exposure to fluorine indicates that fluoride is absorbed under these conditions (Stokinger 1949). No information on absorption rate or extent is available.

Hydrogen Fluoride. A study in rats suggests that hydrogen fluoride is absorbed primarily by the upper respiratory tract, and that removal of hydrogen fluoride from inhaled air by the upper respiratory tract approaches 100% for exposures that range from 30 to 176 mg fluoride/m^3 (Morris and Smith 1982). Furthermore, it is apparent that distribution to the blood is rapid. Immediately following 40 minutes of intermittent exposure, plasma fluoride concentrations correlated closely (correlation coefficient=0.98; $p<0.01$) with the concentration of hydrogen fluoride in the air passed through the surgically isolated upper respiratory tract. Plasma levels were not measured at time points <40 minutes.

Hydrogen Fluoride and Fluoride Dusts. The absorption in humans of inhaled hydrogen fluoride and fluoride dusts was demonstrated by Collings et al. (1952). Their study was conducted on two subjects exposed in the laboratory to an atmospheric concentration of 5.0 mg fluoride/m^3 as hydrogen fluoride during an 8-hour period. Absorption of fluoride was evaluated by monitoring urinary excretion of fluoride during and after exposure. Analysis of 2-hour serial urine samples showed a peak fluoride level

2–4 hours after cessation of exposure, which decreased to base levels within 12–16 hours after exposure. Similar results were obtained using the same protocol to measure urinary fluoride following exposure to air containing 5.0 mg fluoride/m^3 as rock phosphate dust (Collings et al. 1951). Another study reported clinical observations of employees in the production of phosphate rock and triple superphosphate (Rye 1961). Three employees were exposed to airborne fluoride (2–4 ppm) composed of approximately 60% dust and 40% hydrogen fluoride gas. Within 2–3 hours after exposure began, urinary fluoride levels increased from 0.5 to 4.0 mg/L and peaked 10 hours (7–8 mg/L) following cessation of exposure. None of the subjects had prior occupational exposure to fluoride. Although these studies demonstrate absorption of fluoride, none measure the extent of fluoride absorption.

The data presented above show that the fluoride ion, as hydrogen fluoride in fluoride-containing dusts, is absorbed by humans and animals following acute inhalation exposure. The degree of absorption in humans has not been determined. However, the demonstration that virtually 100% of airborne hydrogen fluoride is deposited in the upper respiratory tract of rats, combined with the appearance of fluoride in the urine of humans within at least 2 hours and in the plasma of rats at least 40 minutes following initiation of exposure, indicates that both forms of fluoride are rapidly and completely absorbed by humans by this route. This conclusion is confirmed by data presented in case reports of systemic effects following inhalation (combined with dermal) exposure to hydrogen fluoride/hydrofluoric acid, as discussed in Sections 3.2.1 and 3.2.3.

Furthermore, although the data presented concern only acute exposures, it is expected that virtually complete absorption would also be observed during long-term exposure to low levels of fluoride in the air.

3.4.1.2 Oral Exposure

Data exist on absorption following oral exposure of humans and animals to fluoride as sodium fluoride, calcium fluoride, and in bone meal. Data on absorption rates exist only for sodium fluoride.

Fluoride. Ingested dietary fluoride is readily absorbed from the gastrointestinal tract as the undissociated hydrogen fluoride molecule by passive absorption (Whitford and Pashley 1984). Since the neutral undissociated molecule can penetrate cell membranes and be absorbed much better than the fluoride ion, decreasing the stomach pH increases absorption. The absorption of soluble fluoride in humans is rapid and extensive (97%) (Carlson et al. 1960a; Ekstrand et al. 1977b, 1983; McClure et al. 1945) with maximum plasma fluoride concentrations attained as early as within 30 minutes following exposure (Ekstrand et al. 1977b).

Absorption of ingested fluoride has been investigated in humans in a number of studies. In a study by Carlson et al. (1960a), oral administration of 1 mg fluoride (as sodium fluoride containing ^{18}F) in 250 mL water resulted in a maximum plasma fluoride concentration of 0.13–0.17 mg/L within 60 minutes. At 150 minutes following exposure, ^{18}F was no longer detected in the stomach. In another study, the plasma fluoride concentration after oral administration of 4.5–10 mg fluoride as sodium fluoride tablets or gelatin capsules to eight subjects peaked within 30 minutes of administration (Ekstrand et al. 1977b). Similar observations were reported in children receiving 0.5 mg fluoride as sodium fluoride tablets in water (Ekstrand et al. 1983). Gastrointestinal absorption of fluoride in five men receiving a diet supplemented with sodium fluoride and calcium fluoride in water and food, and bone meal and cryolite in food was determined over a 5-day period (McClure et al. 1945). Fecal excretion data indicated that sodium fluoride in food and water, and calcium fluoride in water were extensively absorbed, while fluoride in bone meal, cryolite, and calcium fluoride in food were not as completely absorbed. About 13–16% of the ingested fluoride was in the feces for the well-absorbed species, while 30–56% of the ingested dose of the poorly-absorbed species appeared in the feces. As described below in Section 3.4.4.2, more recent data indicate that a smaller percent of a sodium fluoride dose appears in the feces than was reported here.

However, additional factors can affect absorption. The absorption of fluoride as calcium fluoride is increased when the material is given with meals (Trautner and Einwag 1987). The suggested explanation was that increased residence time in the upper gastrointestinal tract increases absorption. Fluoride is more completely absorbed from liquids than from solid foods (McClure et al. 1945; Trautner and Siebert 1986). Concurrent ingestion of other salts can increase or decrease absorption. Ingestion of 1,320 mg calcium/day as calcium carbonate reduced the absorption of fluoride (30 mg/day as sodium fluoride, or 0.42 mg fluoride/kg/day) by 22% (Jowsey and Riggs 1978). This result could be due either to inhibition of absorption by calcium, such as due to the insolubility of calcium fluoride, or due to the alkalizing effect of the carbonate. Magnesium (Spencer et al. 1978b) and aluminum antacids (Spencer et al. 1980a, 1980b) decreased absorption in humans. In Sprague-Dawley rats, calcium and magnesium decreased absorption, while phosphate and sulfate increased absorption (Stookey et al. 1964; Weddle and Muhler 1954). Aluminum also decreased absorption in Sprague-Dawley rats (Weddle and Muhler 1954). The effects of salts on fluoride absorption is discussed further in Section 3.11.

Absorbed fluoride is likely to be passed on to the developing human fetus. Placental accumulation of fluoride in humans has been demonstrated following consumption of drinking water containing 0.55 ppm fluoride (Gedalia et al. 1961). Furthermore, the fluoride concentration in the placenta (0.15 ppm) was higher than that in maternal blood (0.09 ppm). Fluoride measurements from maternal uterine vessels and umbilical blood at caesarean section revealed no difference between maternal and fetal levels (Armstrong et al. 1970). However, a partial placental barrier may exist at high maternal fluoride levels (Gedalia 1970). The use of fluoride supplements markedly increased placental fluoride levels, while fluoride

levels in fetal blood remained almost constant. Placental transfer of fluoride to the developing fetus has been demonstrated in rats (Theuer et al. 1971). A high dietary level of fluoride (10 mg fluoride/kg/day) administered to pregnant rats as sodium fluoride resulted in significantly higher fluoride levels in fetuses than in the placenta.

Soluble fluorides are also rapidly and extensively absorbed from the gastrointestinal tract of animals. Rats were administered 0.2 mg fluoride (0.57 mg/kg/day) as sodium fluoride in solution, and absorption was monitored at time points up to 90 minutes. Half of the dose was absorbed with 30 minutes and 86% of the dose was absorbed within 90 minutes (Zipkin and Likins 1957). Oral administration of radio-labeled fluoride (0.08 mg fluoride/kg) to male rats resulted in 89–90% absorption after 8–10 hours (Ericsson 1958).

In summary, existing data indicate that all common forms of inorganic fluoride are rapidly and extensively absorbed by humans. However, there are differences in the extent of absorption between different forms of fluoride and between fluoride in solution and fluoride incorporated in food, and the presence of other ions can affect absorption. The highest degree of absorption (virtually 100%) is seen with aqueous solutions of sodium fluoride. Evidence from humans and animals indicates that absorption begins quickly following ingestion, with studies in animals showing absorption beginning as early as 30 minutes following exposure. Furthermore, the absorbed fluoride is passed to the human fetus during pregnancy.

Most of the existing studies examine acute absorption of fluoride, and there is no indication that absorption of fluoride would be less extensive following low-level, long-term exposure. In the absence of such data, it is expected that absorption would be virtually complete following chronic oral exposure to low levels of most soluble forms of fluoride.

3.4.1.3 Dermal Exposure

Data exist on dermal absorption of hydrofluoric acid in humans and animals, and limited quantitative rate data are available in animals.

Fluorine. Systemic effects have been observed following whole-body exposure to fluorine (Keplinger and Suissa 1968; Stokinger 1949). However, these effects are likely to be due to inhalation exposure, rather than dermal exposure.

Hydrofluoric Acid. Dermal application of hydrofluoric acid results in rapid penetration of the fluoride ion into the skin. Sufficiently large amounts cause necrosis of the soft tissue and decalcification and

corrosion of bone in humans (Browne 1974; Dale 1951; Dibbell et al. 1970; Jones 1939; Klauder et al. 1955). Systemic fluoride poisoning has been reported following accidental dermal exposure to anhydrous hydrogen fluoride (Buckingham 1988; Burke et al. 1973). Although the extent of the contribution of inhalation exposure in these cases is not known, the reports suggest that hydrogen fluoride is quickly absorbed into the body following dermal exposure. However, these studies did not provide useful information concerning the extent of fluoride absorption, or information on absorption of smaller doses.

Dermal absorption of hydrofluoric acid in albino mice of the d.d. strain was inferred in a study by Watanabe et al. (1975). Mice were painted with 0.02 mL of 50% hydrofluoric acid, and the residual acid was wiped off after 5 minutes. The mice were then injected intraperitoneally with [^{14}C]glucose and analyzed by whole body radiography. Radioactivity levels in the liver, renal cortex, lungs, and blood were elevated 30 minutes after injection. This suggests that fluoride was absorbed through the skin and interfered with the tissue distribution of glucose. No data were located on the extent of absorption of fluoride in animals exposed dermally to hydrofluoric acid.

These studies indicate that fluoride as hydrofluoric acid is absorbed through the skin in humans and animals. However, the degree of absorption is not known, nor is it known whether other forms of fluoride would be absorbed, and to what extent. Furthermore, it is expected that the relationship between duration or concentration and degree of absorption would be affected by the corrosive action of hydrofluoric acid. Therefore, prediction of the extent of absorption following exposure to a low concentration of hydrofluoric acid cannot be made based on the existing data.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Fluorine. No data were located regarding the distribution of fluoride following the inhalation exposure of humans to fluorine. In rats exposed to 25 mg/m^3 fluorine for about 5 hours/day, 6 days/week for 21 days, markedly elevated fluoride levels were observed in teeth and bone, the only tissues that were analyzed (Stokinger 1949). Tooth fluoride levels were about 14 times the levels in controls and fluoride levels in the femur were about 6 times those in the controls. Similar concentration-related increases in bone and tooth fluoride levels were observed at the lower concentrations (3 and 0.8 mg/m^3).

Hydrogen Fluoride. No data were located regarding the distribution of fluoride in humans following exposure to only hydrogen fluoride. Evidence from studies in animals supports the inference from occupational studies of exposure to hydrogen fluoride and fluoride dust that fluoride is distributed to the

rest of the body when inhaled. Duration- and concentration-related increases in tooth and bone fluoride levels were reported in the rat following exposure to 7 or 24 mg/m^3 for 6 hours/day, 6 days/week for up to 30 days (Stokinger 1949). Fluoride levels in new bone were up to twice the levels in old bone. The distribution of the fluoride ion was studied in the tissues of rabbits, a guinea pig, and a monkey exposed to hydrogen fluoride at various concentrations (1.5–1,050 mg/m^3) and exposure times (Machle and Scott 1935). The observation period ranged from 9 to 14 months. As might be expected, based on the following discussion of human occupational exposure to fluoride compounds, the fluoride ion accumulated chiefly in the skeleton of all three species.

Several studies in animals have demonstrated that fluoride is widely available through the blood, although actual concentrations in tissues other than blood have, for the most part, not been reported. For example, whole body exposure of male rats to levels ranging from 11 to 116 mg fluoride/m^3 as hydrogen fluoride for 6 hours resulted in a dose-dependent increase in lung and plasma fluoride concentrations (Morris and Smith 1983). In another study, rats exposed to 84 mg fluoride/m^3 as hydrogen fluoride by whole body exposure had significantly elevated levels of fluoride in plasma and lungs 6 hours postexposure (Morris and Smith 1983).

Intermittent high level exposures may result in greater accumulation of fluoride in bones and teeth than continuous exposure. Daily exposure of rats to airborne concentrations of 8 mg hydrogen fluoride/m^3 for a total of 124 hours resulted in a fluoride content of pooled teeth and bone 1.5–1.8 times the fluoride content of similarly pooled teeth and bones in a group exposed to the same concentration on alternate days for a total of 62 hours (Stokinger et al. 1950). If exposure durations were simply additive, the ratio between fluoride concentrations in teeth and bone at the two durations would be expected to be 2.0, rather than 1.5–1.8.

Hydrogen Fluoride and Fluoride Dusts. Limited information was located on the distribution of inhaled fluoride in humans. However, reports of skeletal fluorosis (Chan-Yeung et al. 1983b; Czerwinski et al. 1988; Kaltreider et al. 1972) and elevated bone fluoride levels (Baud et al. 1978; Boivin et al. 1988) after occupational exposure to hydrogen fluoride and fluoride dusts indicate that fluoride is distributed to bone and accumulates there.

Fluoride deposition in bone occurs mainly in regions undergoing active ossification or calcification. If the source of fluoride exposure has been removed, fluoride levels in bone decrease as the bone undergoes remodelling. Areas of fluoride deposition during high-level exposure are distinguished by highly elevated fluoride levels even after the average fluoride level of the bone has returned to normal (Baud et al. 1978).

Studies located indicate that the fluoride ion is distributed rapidly in the bloodstream following inhalation exposure. Evidence in humans and animals indicates that fluoride may be preferentially distributed to bones and teeth following inhalation exposure.

3.4.2.2 Oral Exposure

Fluoride. Ingested soluble fluoride is rapidly absorbed and distributed in humans. Epigastric counts were monitored by Carlson et al. (1960a) in subjects who consumed sodium fluoride containing ^{18}F in water (250 mL at 1 mg/L). Two-and-a-half hours after dosing, the remaining epigastric (abdominal) counts were attributable to fluoride in the spine. Counts in contracted biceps declined 50 minutes after ingestion and were undetectable after approximately 4 hours. In contrast, counts in the femur declined only 15% from their peak value (at 50 minutes) after 4 hours.

Teeth and bone readily take up fluoride following oral exposure (Machle and Largent 1943; McClure and Likins 1951; Suttie et al. 1958). While the rate of fluoride uptake in human teeth may decrease with age (Jackson and Weidmann 1959), it is apparent that the total fluoride content of teeth and bone increases throughout life, and that the amount deposited is dependent on the exposure concentration. A linear relationship was observed between subject age and fluoride concentration in bone ash of lifetime residents of an area with a drinking water supply containing 0.06 ppm fluoride, indicating that bone fluoride levels increase with duration of exposure (Smith et al. 1953). A linear relationship was also observed between the concentration of fluoride in drinking water (ranging from 0.1 to 4 ppm) and the concentration in bone at autopsy in adult humans who had ingested the drinking water (Zipkin et al. 1958). Average fluoride levels in the iliac crest bone ash in people with drinking water fluoride levels of #0.3, 1, and 4 ppm were 700, 2,300, and 6,900 ppm, respectively.

Long-term retention and accumulation of fluoride are primarily confined to calcified tissue in humans (Wagner et al. 1958). Soft tissue concentrations of fluoride do rise transiently following ingestion of fluoride (Carlson et al. 1960b; Hein et al. 1956). Examination of autopsy samples from 23 individuals who had lived in an area where drinking water contained 1–4 ppm fluoride revealed no significant accumulation of fluoride in the heart, liver, lung, kidney, or spleen (Smith et al. 1960). Fluoride concentration in the aorta did increase with age; this was probably associated with increased calcification of the aorta with age. Kidney fluoride levels can be much greater than the levels in plasma (Whitford and Taves 1973).

Fluoride is redistributed as it is released during bone remodelling. The short-term kinetics of this process was investigated in humans by supplementing a diet of 4.4 mg fluoride/day with 9.1 mg fluoride/day as sodium fluoride for 32 days (Spencer et al. 1975b). Urinary fluoride excretion was elevated during the

period of sodium fluoride supplementation, but dropped rapidly after it was removed. Urinary excretion returned to the presupplemented levels within 12 days. Only 9.1% of the retained fluoride was excreted during this period. The progressive decrease over many years of bone fluoride concentrations of skeletal fluorosis patients who have been removed from the source of exposure indicates that there is a second, slower phase of fluoride redistribution (Boivin et al. 1988). Limited data suggest that this phase reduces fluoride levels by one-half in 20 years (Baud et al. 1978). This slower phase may correspond to remodelling of the trabecular bone (WHO 1984).

A limited number of studies were located that determined the distribution of fluoride in animal tissues following oral exposure. For example, in a lamb sacrificed 2 hours after a one-time ingestion of sodium fluoride containing ^{18}F, the absorbed fluoride was found to be widely distributed in the blood, bile, muscle, spleen, pancreas, liver, lymph node, and skeleton (Perkinson et al. 1955). These results are consistent with a short half-life in soft tissues.

In a study of rats exposed to 0.1% sodium fluoride in their diet for up to 113 days, analysis of fluoride content in the bone at various intervals revealed rapid uptake of the fluoride into bone (Suttie and Phillips 1959). However, final fluoride levels in bone were inversely correlated with the initial ages of the rats, suggesting that the rate of fluoride uptake decreases with age.

Evidence from one animal study indicates that fluoride deposits in bone are released as the bone undergoes normal absorption and redeposition (Guo et al. 1988). Weanling rats were exposed to high levels of fluoride (50 mg fluoride/L) in drinking water for 3 weeks to establish a baseline fluoride level in bone. Fluoride levels in bone were determined following exposure to a low-fluoride diet or a low-calcium, low-fluoride diet. Comparison of control fluoride-treated rats to those fed low calcium diets (which accelerates bone resorption and deposition) demonstrated that approximately 40% of the fluoride mobilized during bone resorption is not redeposited in the skeleton (Guo et al. 1988). About 30% of the bone fluoride was lost in a 4-week period. Presumably, the fluoride that is not redeposited is excreted. The high level of fluoride loading in the bone make it difficult to compare this study with results from studies in humans.

In a short-term distribution kinetic study in rats, Whitford et al. (1979a) found that soft tissues do not strongly bind fluoride, and that most of these tissues are kinetically homogeneous with plasma. Furthermore, the blood brain barrier is effective in preventing fluoride migration into the central nervous system; brain tissue fluoride concentrations did not exceed 10% of plasma concentrations following intravenous administration. It has also been shown that fecal excretion of fluoride can exceed dietary intake when the diet is supplemented with calcium (Whitford 1994); under these conditions, fluoride balance (the difference between total intake and total excretion) is negative.

Fluoride accumulates at least temporarily in the soft tissues of dairy cows; however, differences between the bovine and human digestive systems preclude firm conclusions based on this information alone. Soft tissue concentrations of fluoride were measured in 20 dairy cows exposed to 0–50 ppm (equivalent to 1.4 mg/kg/day) fluoride as sodium fluoride in the feed for 5.5 years (Suttie et al. 1958). Cows exposed to 50 ppm fluoride had residues of fluoride mainly in the pancreas (4.2 ppm), kidney (19.3 ppm), and whole blood (0.67 ppm). As in humans, bone-fluoride concentration corresponded to the amount of fluoride ingested.

Existing literature in humans indicates that continuous fluoride exposure results in a build-up of fluoride levels in bone and teeth. Furthermore, fluoride levels in bone are related directly to the level of steady state intake. With the exception of the aorta (Smith et al. 1960) and kidney (Whitford and Taves 1973), there is no evidence of accumulation or retention of fluoride in soft tissues in humans. Upon cessation of exposure, fluoride levels in bone are expected to decrease slowly; however, the time period over which this would occur in humans is not known.

3.4.2.3 Dermal Exposure

No information was located in humans or animals regarding the distribution of fluorine, hydrogen fluoride, or fluoride following dermal absorption.

3.4.2.4 Other Routes of Exposure

Based on the results of a five-compartment computer model, Charkes et al. (1978) calculated that about 60% of intravenously administered fluoride is taken up by bone and the half-time for this uptake is about 13 minutes.

Perkinson et al. (1955) found initial rates of removal of fluoride from sheep and cow blood to be 41 and 32%/minute of the intravenously administered dose, respectively. These data suggest a rapid distribution of fluoride and corroborate findings reported by other routes of administration.

Fluoride distribution in rats was examined during and after continuous intravenous infusion of radiolabeled sodium fluoride at varying chemical dose rates for 3 hours (Knaus et al. 1976). Blood, kidneys, and lungs contained the highest fluoride concentrations at doses up to 3.6 mg fluoride/kg/hour, but at 6 mg/kg/hour, the fluoride content of the liver, spleen, and hollow organs increased sharply, indicating that the dose exceeded the amount readily processed by the excretory mechanisms of the body. In rat pups injected intraperitoneally with 0.1 µg fluoride/g body weight as sodium fluoride solution, significant increases in the fluoride content occurred in the developing enamel and bone (Bawden et

al. 1987). Thus, regardless of the route of administration, some fluoride is deposited in teeth, bone, and soft tissues of animals, and some is excreted in the urine, sweat, and saliva.

3.4.3 Metabolism

Fluoride is believed to replace the hydroxyl ion (OH^-) and possibly the bicarbonate ion (HCO_3^-) associated with hydroxyapatite—a mineral phase during formation of bone (McCann and Bullock 1957; Neuman et al. 1950). The resultant material is hydroxyfluorapatite. Once absorbed, a portion of the fluoride is deposited in the skeleton, and the remainder is excreted in the urine, feces, sweat, and saliva within 24 hours (Dinman et al. 1976a, 1976b; McClure et al. 1945). Thus, skeletal sequestration and renal excretion are the two major means by which the body prevents circulation of toxic amounts of fluoride ion (Hodge 1961). Urinary excretion is markedly decreased in the presence of decreased renal function (Kono et al. 1984).

The fluoride ion carried in human blood serum exists in two forms, namely as an inorganic ion F^- and in combination with an organic molecule (Halton et al. 1984). The toxicological significance, if any, of the latter form is unknown. A portion of the circulating inorganic fluoride acts as an enzyme inhibitor because it forms metal-fluoride-phosphate complexes that interfere with the activity of those enzymes requiring a metal ion cofactor. In addition, fluoride may interact directly with the enzyme or the substrate. It is a general inhibitor of the energy production system of the cell (i.e., glycolytic processes and oxidative phosphorylation enzymes responsible for forming ATP) (Guminska and Sterkowicz 1975; Najjar 1948; Peters et al. 1964; Slater and Bonner 1952). Although much is known about enzyme inhibition by fluoride, the human health significance remains to be determined. The studies on enzymatic inhibition by fluoride were *in vitro* studies and used fluoride concentrations that were significantly higher than concentrations that would be normally found in human tissues.

In rats exposed to 84 mg fluoride/m^3 as hydrogen fluoride by whole body exposure an average of 90% of recovered fluorine was nonionized (Morris and Smith 1983). This observation indicates that most of the fluorine in the plasma F-fraction of fluoride-exposed animals is in the form of nonionizable fluorine.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Fluorine. No data were located regarding excretion of fluoride following human inhalation exposure to fluorine. Urinary fluoride levels were increased in dogs and rabbits exposed to levels as low as 0.8 mg/m^3 for 5–6 hours/day, 6 days/week for 35 days (Stokinger 1949). No quantitative data were reported at this level, but urinary fluoride levels in rabbits exposed to 3 mg/m^3 were 1.5 times normal. No further details were reported.

Hydrogen Fluoride. Studies in humans indicate that fluoride absorbed from inhaled hydrogen fluoride over an 8-hour work shift is excreted even during exposure, with urinary excretion peaking approximately 2–4 hours after cessation of exposure (about 10 hours following beginning of exposure) (Collings et al. 1951; Rye 1961). These studies have been discussed in Section 3.3.1.

Overnight urinary fluoride excretion in dogs and rabbits exposed to 7 mg/m^3 hydrogen fluoride for 6 hours/day, 6 days/week for 30 days was about 1.5 times that of controls (Stokinger 1949). No further details were reported.

3.4.4.2 Oral Exposure

Fluoride. The principal route of excretion of ingested fluoride is via the urine as demonstrated in a variety of species. In general, urine accounts for about 50–70% of the fluoride intake and feces accounts for 5–10% (Machle and Largent 1943; Spencer et al. 1970). Estimates of total excretion range from about 50% (Spencer et al. 1970) to about 100% (McClure et al. 1945). These varying estimates lead to widely varying estimates of the amount of fluoride that is stored in the body. About 1% of fluoride intake is excreted into saliva (Carlson et al. 1960a), although since saliva is swallowed, this amount does not enter mass balance calculations. In species other than humans, there is little published information relating ingested fluoride concentrations and urine fluoride concentrations over prolonged periods.

There is a striking linear relationship between the concentration of fluoride in drinking water and in the urine of humans exposed continuously to fluoride. However, plasma fluoride levels are reflected better by the urinary fluoride excretion rate than by the concentration of fluoride in the urine (Ekstrand and Ehrnebo 1983). Large amounts of fluoride were excreted for prolonged periods by persons who lived for many years in areas with high fluoride water levels and who subsequently moved to areas with low fluoride levels, which indicated the excretion of fluoride that was mobilized from bone (Likins et al. 1962). Individuals who had been chronically exposed to a drinking water supply containing 1 mg

fluoride/L (1 ppm) and then received a single 5 mg dose of fluoride as sodium fluoride began excreting increased amounts of fluoride in their urine <2 hours following exposure (Zipkin and Leone 1957). Total 24-hour fluoride intake was estimated at 8.1 mg. Within 3 hours, 20% (1.6 mg fluoride) of the fluoride was excreted in the urine; 54% (4.4 mg) was excreted in the urine within 24 hours. By 9 hours after the fluoride bolus, the urinary fluoride excretion rate had almost returned to the preexposure rate of about 0.1 mg/hour. In persons not occupationally exposed to fluoride and not using water containing added fluoride, fecal elimination is usually <0.2 mg/day (NAS 1971a).

Urinary fluoride excretion generally corresponds to 50–70% fluoride intake, depending on a number of factors (Machle and Largent 1943; Spencer et al. 1970). In one study, baseline fluoride balance was determined by monitoring fluoride in diet and water, and measuring excretion in urine and feces in 10 people for four 6-week periods (Spencer et al. 1970). Perspiration was not monitored. Average fluoride intake was 4.4 mg/day, of which an average of 1.8 mg/day (41%) was retained (range 1.6–2.2 mg/day). An average of 51% of the intake was excreted in urine, and 6.6% was excreted in feces. The diet was then supplemented with 9 mg fluoride/day as sodium fluoride for 30 days, and monitored as before. Average urinary and fecal excretion as a percent of intake were comparable to the levels found in the baseline study (54 and 6.4%, respectively). However, the ranges were larger during the increased fluoride intake. During supplementation, intake exceed excretion by an average of 5.4 mg/day (39% of intake). After the sodium fluoride supplementation was removed, retention of ingested fluoride dropped to an average of 32% as stored fluoride was cleared from the body. In human subjects consuming 6 mg fluoride daily in the diet (equivalent to 0.09 mg/kg/day), about half of the absorbed fluoride was excreted in the urine (Machle and Largent 1943). Of 10 subjects with endemic fluorosis who ingested 8–18 mg fluoride/day, the fluoride balance ranged from an excess excretion of 30% to excretion that was 40% less than intake (Jolly 1976). In another study, total fluoride intake was monitored in a healthy male subject who consumed his normal diet, and was found to be about 0.5 mg/day (Machle et al. 1942). Fluoride excretion in feces and urine was monitored and determined to be within 5% of intake. This study is limited by the use of only one subject.

Using radiolabeled fluoride, Carlson et al. (1960a) demonstrated that 51 and 63% of the fluoride filtered by the kidney was reabsorbed in two human subjects, respectively. The inefficiency of the human kidney in reabsorbing filtered fluoride results in the rapid urinary excretion of fluoride. Fluoride excretion is decreased in acidic urine, probably due to reabsorption of nondissociated hydrogen fluoride (Ekstrand et al. 1980b). The renal fluoride clearance rate is lower in children than in adults (Spak et al. 1985). Although this study was conducted in children with suspected kidney disease or suspected renal dysfunction, the conclusion was reached based on children with normal glomerular filtration rates. Urinary fluoride concentration is markedly lower in children than in adults, and increases with age from

ages 1–6-years (Gdalia 1958). These studies indicate that children store more fluoride than adults do, due to high uptake in developing bones.

Sweat is a route of fluoride elimination. In a 1945 study, subjects who ingested 3.7 mg fluoride in 1 day, of which 3 mg was from naturally fluoridated water or water to which sodium fluoride had been added, excreted about 19% of the ingested fluoride in sweat under comfortable conditions (McClure et al. 1945). Under hot-moist conditions, the excretion in sweat increased to 42%. Although water ingestion increased, the provided drinking water was low fluoride, so fluoride retention decreased. In the only other study located where fluoride in sweat was measured, up to 50% of fluoride excretion under hot conditions was in sweat. Both studies were compromised by the small number of subjects.

Limited data were located on excretion of ingested fluoride in animals. The data support the observations in humans that fluoride is rapidly excreted in urine. This was demonstrated by Chen et al. (1956), who measured renal clearance of fluoride in female dogs. In dogs receiving drinking water containing fluoride at 1 ppm, the renal fluoride clearance was 2.7 mL/minute, and the fluoride:chloride clearance ratio was 19:1.

Evidence from studies in humans and animals demonstrates that excretion of ingested fluoride occurs primarily in the urine, and to a lesser extent in the feces, sweat, and saliva. This excretion is rapid, occurring over a period of hours (McClure et al. 1945; Spencer et al. 1970). As discussed previously, a portion of the absorbed fluoride is sequestered in bone. Continued secondary excretion of this pool of fluoride is expected based on animal studies. It is also expected that this excretion would occur in the urine.

3.4.4.3 Dermal Exposure

No studies were located regarding excretion of fluorine, hydrogen fluoride, or fluoride in humans or animals following dermal exposure. However, in the absence of evidence to the contrary, it is expected that dermally absorbed fluoride would be sequestered in bone and excreted in urine in a manner similar to that observed following oral or inhalation exposure.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of

potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste

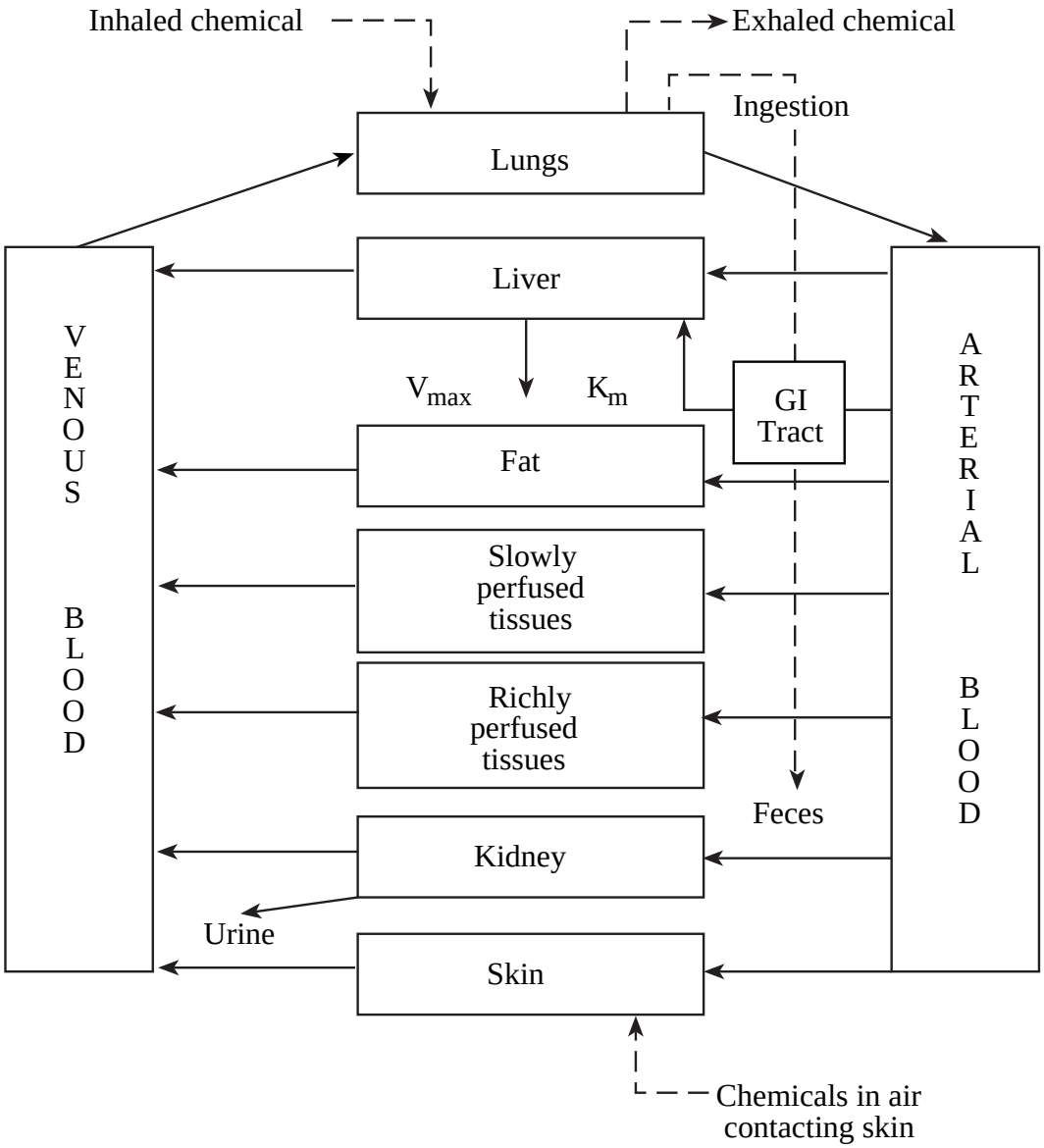
sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

Only one published PBPK model has been identified (Rao et al. 1995); it differs from published compartmental models for fluoride kinetics (Charkes et al. 1978, 1979; Hall et al. 1977) in that the earlier models were data-based and useful only to simulate short-term fluoride kinetics. Because the fluoride ion is characterized by its long residence time in the body, health effects based on long-term fluoride exposure are of concern. In contrast to the earlier models, the Rao et al. PBPK model is amenable to extrapolation across species, routes, and doses, thereby offering an advantage in quantitative risk assessment for fluoride exposure.

In order to assess the complex relationship between extended fluoride exposure, target tissue (bone) dose, and tissue response, a sex-specific PBPK model has been developed to describe the absorption, distribution, and elimination of fluorides in rats and humans (Rao et al. 1995). The PBPK model incorporates age and body weight dependence of the physiological processes that control the uptake of fluoride by bone and the elimination of fluoride by the kidneys. Six compartments (lung, liver, kidney, bone, and slowly- and rapidly-perfused compartments) make up the model. The bone compartment includes two subcompartments: a small, flow-limited, rapidly exchangeable surface bone compartment, and a bulk, virtually nonexchangeable inner bone compartment. The inner bone compartment contains nearly all of the whole body content of fluoride, which, in the longer time frame, may be mobilized through the process of bone modeling and remodeling. This model has been validated by comparing predictions with experimental data gathered in rats and humans after drinking water and dietary ingestion of fluoride.

The PBPK model permits the analysis of the combined effect of ingesting and inhaling fluorides on the target organ, bone. It takes into account the effects of age and growth; in the human model, for instance, the bone and renal clearance rates accounted for 90 and 10%, respectively, during the growth period, compared to about 50% each in adulthood. Estimates of fluoride concentrations in bone are calculated and related to chronic fluoride toxicity. The model incorporates nonlinear binding rates of fluoride to bone, which has been described at high plasma concentrations. The model is thus useful for predicting some of the long-term metabolic features and tissue concentrations of fluoride that may be of value in understanding positive or negative effects of fluoride on human health. In addition, the PBPK model provides a basis for cross-species extrapolation of the effective fluoride dose at the target tissue (bone) in the assessment of risk from different exposure conditions.

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

3.5 MECHANISMS OF ACTION

The following section on the mechanism of fluoride prevention of dental caries is excerpted from the Centers for Disease Control and Prevention's report entitled "Recommendations for using fluoride to prevent and control dental caries in the United States" (DHHS 2001b):

> Dental caries is an infectious, transmissible disease in which bacterial by-products (i.e., acids) dissolve the hard surfaces of teeth. Unchecked, the bacteria can penetrate the dissolved surface, attack the underlying dentin, and reach the soft pulp tissue. Dental caries can result in loss of tooth structure, pain, and tooth loss and can progress to acute systemic infection.
>
> Cariogenic bacteria (i.e., bacteria that cause dental caries) reside in dental plaque, a sticky organic matrix of bacteria, food debris, dead mucosal cells, and salivary components that adheres to tooth enamel. Plaque also contains minerals, primarily calcium and phosphorus, as well as proteins, polysaccharides, carbohydrates, and lipids. Cariogenic bacteria colonize on tooth surfaces and produce polysaccharides that enhance adherence of the plaque to enamel. Left undisturbed, plaque will grow and harbor increasing numbers of cariogenic bacteria. An initial step in the formation of a carious lesion takes place when cariogenic bacteria in dental plaque metabolize a substrate from the diet (e.g., sugars and other fermentable carbohydrates) and the acid produced as a metabolic by-product demineralizes (i.e., begins to dissolve) the adjacent enamel crystal surface. Demineralization involves the loss of calcium, phosphate, and carbonate. These minerals can be captured by surrounding plaque and be available for reuptake by the enamel surface. Fluoride, when present in the mouth, is also retained and concentrated in plaque.
>
> Fluoride works to control early dental caries in several ways. Fluoride concentrated in plaque and saliva inhibits the demineralization of sound enamel and enhances the remineralization (i.e., recovery) of demineralized enamel (Featherstone 1999; Koulourides 1990). As cariogenic bacteria metabolize carbohydrates and produce acid, fluoride is released from dental plaque in response to lowered pH at the tooth-plaque interface (Tatevossian 1990). The released fluoride and the fluoride present in saliva are then taken up, along with calcium and phosphate, by de-mineralized enamel to establish an improved enamel crystal structure. This improved structure is more acid resistant and contains more fluoride and less carbonate (Chow 1990; Ericsson 1977; Featherstone 1999; Kidd et al. 1980; Thylstrup 1990; Thylstrup et al. 1979). Fluoride is more readily taken up by demineralized enamel than by sound enamel (White and Nancollas 1990). Cycles of demineralization and remineralization continue throughout the lifetime of the tooth.
>
> Fluoride also inhibits dental caries by affecting the activity of cariogenic bacteria. As fluoride concentrates in dental plaque, it inhibits the process by which cariogenic bacteria metabolize carbohydrates to produce acid and affects bacterial production of adhesive polysaccharides (Hamilton 1990). In laboratory studies, when a low concentration of fluoride is constantly present, one type of cariogenic bacteria, Streptococcus

mutans, produces less acid (Bowden 1990; Bowden et al. 1982; Marquis 1990; Rosen et al. 1978). Whether this reduced acid production reduces the cariogenicity of these bacteria in humans is unclear (Van Loveren 1990).

Saliva is a major carrier of topical fluoride. The concentration of fluoride in ductal saliva, as it is secreted from salivary glands, is low --- approximately 0.016 parts per million (ppm) in areas where drinking water is fluoridated and 0.006 ppm in nonfluoridated areas (Oliveby et al. 1990). This concentration of fluoride is not likely to affect cariogenic activity. However, drinking fluoridated water, brushing with fluoride toothpaste, or using other fluoride dental products can raise the concentration of fluoride in saliva present in the mouth 100- to 1,000-fold. The concentration returns to previous levels within 1--2 hours but, during this time, saliva serves as an important source of fluoride for concentration in plaque and for tooth remineralization (Rölla and Ekstrand 1996).

Applying fluoride gel or other products containing a high concentration of fluoride to the teeth leaves a temporary layer of calcium fluoride-like material on the enamel surface. The fluoride in this material is released when the pH drops in the mouth in response to acid production and is available to remineralize enamel (LeGeros 1990).

In the earliest days of fluoride research, investigators hypothesized that fluoride affects enamel and inhibits dental caries only when incorporated into developing dental enamel (i.e., preeruptively, before the tooth erupts into the mouth) (Dean et al. 1935; McClure and Likins 1951). Evidence supports this hypothesis (Groeneveld et al. 1990; Marthaler 1979; Murray 1993), but distinguishing a true preeruptive effect after teeth erupt into a mouth where topical fluoride exposure occurs regularly is difficult. However, a high fluoride concentration in sound enamel cannot alone explain the marked reduction in dental caries that fluoride produces (Levine 1976; Margolis and Moreno 1990). The prevalence of dental caries in a population is not inversely related to the concentration of fluoride in enamel (Clarkson et al. 1996), and a higher concentration of enamel fluoride is not necessarily more efficacious in preventing dental caries (Arends and Christoffersen 1990).

The laboratory and epidemiologic research that has led to the better understanding of how fluoride prevents dental caries indicates that fluoride's predominant effect is posteruptive and topical and that the effect depends on fluoride being in the right amount in the right place at the right time. Fluoride works primarily after teeth have erupted, especially when small amounts are maintained constantly in the mouth, specifically in dental plaque and saliva (Clarkson et al. 1996). Thus, adults also benefit from fluoride, rather than only children, as was previously assumed.

A number of mechanisms are involved in the toxicity of fluoride to bone. Fluoride ions are incorporated into bone by substituting for hydroxyl groups in the carbonate-apatite structure to produce hydroxyfluorapatite, thus altering the mineral structure of the bone (Chachra et al. 1999). Unlike hydroxyl ions, fluoride ions reside in the

plane of the calcium ions, resulting in a structure that is electrostatically more stable and structurally more compact (Grynpas 1990). Following administration of fluoride, there is a shift in the mineralization profile towards higher densities and increased hardness (Chachra et al. 1999). However, the structure of the bone (cortical thickness and the trabecular architecture of the femoral head) was largely unchanged in rabbits by fluoride administration. Chachra et al. (1999) suggest that the shift in mineralization could be due to either hypermineralization of older (denser) fractions or to a greater packing density of the hydroxyapatite crystals. Although fluoride administration is associated with an increase in bone mass, *in vivo* and *in vitro* animal studies have found a negative association between fluoride-induced new bone mass and bone strength, suggesting that the quality of the new bone was impaired by the fluoride (Silva and Ulrich 2000; Turner et al. 1997). Because bone strength is thought to derive mainly from the interface between the collagen and the mineral (Catanese and Keavney 1996), alteration in mineralization probably affects strength. The wider crystals, which are formed after fluoride exposure, are presumably not associated with collagen fibrils and thus, do not contribute to mechanical strength. Turner et al. (1997) found that the crystal width was inversely correlated with bending strength of the femur. Thus, although there is an increase in hardness and bone mass and unaltered structure, the mechanical strength of bone is decreased (Cachra et al. 1999).

In addition to the physicochemical effect of fluoride on the bone, at high doses, fluoride can be mitogenic to osteoblasts (Farley et al. 1990; Gruber and Baylink 1991) and inhibitory to osteoclasts. The osteoblasts are still active, although there are fewer plump, cuboidal, highly secretory osteoblasts; whereas fluoride is mitogenic to osteoblastic precursors (Bonjour et al. 1993), it is toxic to individual osteoblasts at the same concentration (Chachra et al. 1999). The effect of fluoride on osteoclasts is not well understood; it appears that fluoride decreases the amount of bone resorbed by osteoclasts (Chachra et al. 1999).

Studies in humans and animals suggest that the effect of fluoride on bone strength is biphasic. In rats administered 1–128 ppm fluoride as sodium fluoride in drinking water for 16 weeks, both increases and decreases in bone strength were found; the maximum femoral bone strength occurred at 16 ppm (Turner et al. 1992). A biphasic relationship between femoral bone strength and bone fluoride content was found. Bone strength increased 18% as the bone fluoride content increased from 100 to 1,216 ppm, and decreased by 31% as the bone fluoride levels increased from 1,216 to 10,000 ppm. It should be noted that the bone fluoride levels in this study, as well as other studies discussed in this section, resulted from high doses of fluoride. Arnala et al. (1986) measured fluoride levels in iliac crest biopsies taken from 18–25 subjects with hip fractures living in areas with low fluoride (<0.3 ppm), high fluoride (>1.5 ppm), or with fluoridated (1.0–1.2 ppm) water. The average fluoride levels in the bone were 450, 3,720, and 1,590 ppm, respectively.

The biphasic nature of bone effects is supported by data from clinical trials in women with postmenopausal osteoporosis (Haguenauer et al. 2000). The meta-analyses of 12 studies found a significant increase in the relative risk of nonvertebral fractures in subjects ingesting high doses of fluoride (>30 mg/day); in subjects administered low fluoride doses or slow-release formulations, there was no effect on nonvertebral fractures.

Similarly, there was no effect on vertebral fracture risk in high fluoride dose subjects, but a decrease in this risk in subjects administered low fluoride doses or slow-release formulations was found.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Thomas (1992) and again by Colborn (1993), was also used in 1996 when Congress mandated the Environmental Protection Agency (EPA) to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Although there is no evidence that fluoride is an endocrine disruptor, there are some data to suggest that fluoride does adversely affect some endocrine glands. An increase in serum thyronine levels, in the absence of changes in triiodothyronine and thyroid stimulating hormone levels, were observed in individuals living in areas of India with high fluoride levels in the drinking water (Michael et al. 1996). In contrast, a decrease in thyroxine levels was observed in rats exposed to fluoride in drinking water for 2 months (Bobek et al. 1976). Significant decreases in serum testosterone have been observed in rats exposed to sodium fluoride for 50–60 days (Araibi et al. 1989; Narayana and Chinoy 1994).

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of

body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

A number of studies have examined the effects of fluoride in children. Due to its cariostatic properties, several agencies (for example, NRC 1999; WHO 1973) advocate fluoride supplementation in children. However, there is a delicate balance between prevention of dental caries and the occurrence of dental fluorosis. Dental fluorosis is characterized by increased porosity of the tooth enamel that may become stained and pitted. In milder forms of dental fluorosis, opaque striations can run horizontally across the surface of the teeth, sometimes becoming confluent giving rise to white opaque patches. The occurrence of mild dental fluorosis is approximately 10–20% in children living in cities with water containing 0.7–1.2 ppm fluoride, which is the recommended range for water fluoride concentration for the prevention of dental caries (DHHS 1991; Heifetz et al. 1988); it is likely that the children were also exposed to other sources of fluoride from manufactured beverages and food. Mild dental fluorosis is considered to be a cosmetic effect and does not appear to affect tooth function. Because dental fluorosis is a response to fluoride exposure during the preeruptive maturation of teeth, only children are susceptible to this effect.

Approximately 99% of the body's fluoride is found in calcified tissues. Chronic exposure to high levels of fluoride results in bone thickening and exostoses (skeletal fluorosis). Because of the dynamic nature of growing bone, it is likely that children will deposit more fluoride in bone than adults consuming an equal amount of fluoride. However, it is not known if children would be more susceptible to skeletal fluorosis than adults.

Developmental effects have been observed in humans and animals exposed to fluoride. In humans, an increased occurrence of spina bifida was found in children living in areas of India with high levels of fluoride in the drinking water (Gupta et al. 1995). However, this study had several deficiencies. For example, it did not address the nutritional status of the mothers. This is important because folic acid deficiency has been implicated in the etiology of spina bifida (Hernandez-Diaz et al. 2001; Honein et al. 2000). In addition, the paper did not provide the fluoride levels in the blood of the mothers, nor radiographic evidence of spina bifida. Studies by Li et al. (1995a) and Lu et al. (2000) concluded that there were decreases in IQ scores in children living in areas of China with high fluoride levels due to soot from coal burning, but it is not known if other contaminants in the soot also contributed to this effect, and the adequacy of the design of these studies is highly questionable. In the Gupta et al. (1995) and Li et al. (1995a) studies, the observed effects occurred in children with dental and/or skeletal fluorosis. In general, developmental effects have not been observed in rat or rabbit oral exposure studies (Collins et al. 1995; Heindel et al. 1996). However, the animal studies did not assess potential neurodevelopmental effects. The available human and animal data suggest that the developing fetus is not a sensitive target of fluoride toxicity.

Fluoride retention appears to be higher in children than adults. Approximately 80% of an absorbed dose of fluoride is retained in young children compared to 50% in adults (Ekstrand et al. 1994a, 1994b). This is supported by the finding that renal fluoride clearance rate and urinary fluoride concentration are markedly lower

in children than adults (Gdalia 1958; Spak et al. 1985). This difference in fluoride retention is due to high fluoride uptake in developing bones. Data on other potential age-related differences in the toxicokinetic properties of fluoride were not located. Only a small proportion of ingested fluoride is transferred from mother to child through breast milk (Ekstrand et al. 1984b; Spak et al. 1982).

Most of the available information on biomarkers, interactions, and methods for reducing toxic effects is from adults and mature animals; no child-specific information was identified, with the exception of biomarker data. It is likely that the available information in adults will also be applicable to children.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to fluorine, hydrogen fluoride, and fluorides are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by fluorine, hydrogen fluoride, and fluorides are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 "Populations That Are Unusually Susceptible".

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Fluorine, Hydrogen Fluoride, and Fluorides

There is extensive literature regarding fluoride levels in biological tissues such as urine, teeth, bone, and fingernails as indices of exposure. Since it does not produce any metabolites, the fluoride ion itself is the measured indicator. The most commonly used medium for identifying fluoride exposure is urinary levels (Ekstrand et al. 1983). Several investigators have used this parameter to detect exposure to sodium fluoride through drinking water (Zipkin et al. 1956) or by ingestion (i.e., toothpaste or diet) (Ekstrand et al. 1983). Occupational exposure to hydrogen fluoride is also evaluated from urine fluoride levels (Yoshida et al. 1978).

Urinary fluoride levels are generally #1 mg/L when the water supply contains #1 ppm fluoride (Schamschula et al. 1985; Venkateswarlu et al. 1971; Zipkin et al. 1956). Only one report was located of urinary fluoride levels following acute poisoning. Following dermal exposure to about 5 g hydrofluoric acid over 2.5% of the body surface (along with concomitant inhalation exposure), the urinary fluoride level in the first sample obtained 3.5 hours after the accident was 87.0 mg/L (Burke et al. 1973). It is difficult to determine urine levels that are associated with chronic effects such as skeletal fluorosis, because no studies that report urinary fluoride levels, accurate exposure levels, duration of exposure, and health effects were located. Probably the most complete study reports average urinary fluoride levels of 9 mg/L following inhalation exposure to 2.4–6.0 mg/m^3 for an unspecified period of time (Kaltreider et al. 1972). Marked evidence of fluorosis was seen in these workers. In another study (Dinman et al. 1976c), the average postshift urinary fluoride level after 3–5 working days was 5.7 mg/L (range, 2.7–10.4). No exposure levels were available, but they were reported to be lower than in the plant where urinary fluoride levels were 9 mg/L. In spite of 10–43 years of occupational exposure, no signs of skeletal fluorosis were seen. This study may provide urinary fluoride levels that are not associated with skeletal fluorosis, but any sensitive workers may have left such work and not been included in the study. These studies are describe in more detail in Section 3.2.1.2.

Urinary fluoride levels up to 13.5 mg/L have been reported in areas of India where skeletal fluorosis due to high water fluoride levels (up to 16.2 ppm) is prevalent (Singh et al. 1963).

Other media that have been used to measure fluoride exposure include plasma (Ekstrand et al. 1983), saliva (Whitford et al. 1999b), and tooth enamel (McClure and Likins 1951). When using saliva as a biomarker, ductal saliva should be obtained under fasting conditions when measuring body burden (long-term intake) of fluoride (Whitford et al. 1999b). Care must be taken when using plasma fluoride as an indicator of exposure; dosage,

time, and duration must be taken into account (Whitford and Williams 1986). The normal plasma fluoride level is related to daily intake of fluoride (Ikenishi et al. 1988; NAS 1971a). A plasma fluoride level of 35.2 mg/L was measured in a case of fatal oral hydrofluoric acid poisoning (Manoguerra and Neuman 1986). No studies regarding normal serum fluoride levels were located, but a level of 2 mg/L was reported in a case of severe oral poisoning with 53 g fluoride as sodium fluoride (Abukurah et al. 1972). Multiple episodes of ventricular fibrillation and tetany occurred, but the patient recovered following stomach lavage and treatment.

The biomarkers mentioned above can be used for acute exposure to fluoride. Concentrations can peak within 1 hour after exposure since fluoride is rapidly absorbed from all routes of exposure. Fluoride salts possess a peculiar "soapy-salty" taste that enables some individuals to recognize that they are consuming large quantities of fluoride. With chronic exposures, such as from drinking water containing fluoride, urinary fluoride levels initially increase, and then reach a constant level. In workers, postshift urinary levels differ from preshift levels since fluoride exposure during the work day is absorbed rapidly into the body. However, these measurements may not always be useful for quantifying chronic exposure because fluoride can accumulate in bones (Carlson et al. 1960a). It may be retained in the skeletal tissues for a long period after the end of exposure, and later re-enter circulating blood to be reabsorbed or excreted in urine. Furthermore, background tissue/fluid levels may affect these measurements since fluoride is prevalent in the environment from dietary sources. Calcium, which is a major element in the body, may interfere with biological fluoride measurements due to its ability to bind fluoride (Richards et al. 1982). This may prevent the quantitation of exposure, because plasma and urine fluoride levels may be unaffected. An important factor in biological fluid fluoride concentration is pH (Ekstrand et al. 1980a). When urine is alkaline, fluoride urine excretion increases and is followed by a decline in plasma fluoride.

Bone fluoride levels can be used to quantitate long term fluoride exposure (Baud et al. 1978; Boivin et al. 1988). However, this requires a bone biopsy, so bone fluoride levels are most frequently measured after clinical signs appear. As described in Section 3.2.2.2, the fluoride level found in bone varies between bones and increases with age. That section also describes fluoride levels in normal bone, and levels associated with various effects.

Studies of Hungarian (Schamschula et al. 1985) or Brazilian (Whitford et al. 1999a) children have demonstrated a direct relationship between fluoride concentrations in drinking water and fluoride levels in fingernail clippings, suggesting that fluoride in fingernails may be a reliable biomarker of exposure.

3.8.2 Biomarkers Used to Characterize Effects Caused by Fluorine, Hydrogen Fluoride, and Fluorides

Because soft tissues do not accumulate significant levels of fluoride over long periods of time, effects of chronic exposure to fluoride first appear in the skeletal system. Chronic oral fluoride exposure can produce dental fluorosis (Duxbury et al. 1982), and higher levels of oral or inhalational exposure can lead to skeletal effects (Kaltreider et al. 1972; Leone et al. 1955). Dental fluorosis is characterized by mottling and erosion of the enamel. Only children are susceptible since their teeth are still developing. Thus, teeth mottling, staining,

erosion, hypoplasia, and excessive wear are possible markers of effect for fluoride exposure (Walton 1988). It should be noted that dental fluorosis develops during tooth formation, a process that occurs over several years. In recent studies, about 22% of children exposed to 0.7–1.2 ppm fluoride in drinking water had very mild to mild dental fluorosis, characterized by small white spots on the teeth (DHHS 1991). Brown spots appeared on the teeth of 7.6% of the children exposed to 2 ppm fluoride in water.

Alteration in bone density or derangement of trabecular structure can be detected by radiographs, and can indicate fluoride-induced changes. However, these are nonspecific changes and can be associated with other exposures. Other metals can sequester in the skeleton, and produce similar changes observed in radiographs. Exostoses, apposition of new bone, ossification of ligaments and tendon insertions, and metastatic aberrant growth of new bone appear to be much more specific and constant findings in fluorosis (Vischer et al. 1970). Skeletal fluorosis has been reported following inhalation exposure to 2.4–6.0 mg/m^3 for an unspecified duration (Kaltreider et al. 1972). As discussed in Section 3.2.2.2, nutritional status plays a large role in determining the oral fluoride exposure levels that lead to this effect. In the few cases of skeletal fluorosis in the United States for which doses are known, they are generally 15–20 mg/day for over 20 years (Bruns and Tytle 1988; Sauerbrunn et al. 1965).

No well-documented information was located regarding biomarkers of effect for fluoride, although there are studies in which cellular changes occurred after fluoride exposure. Increases in glucose or lipid metabolism have been reported in tissues after exposure to fluorides (Dousset et al. 1984; Shearer 1974; Watanabe et al. 1975). Changes in erythrocyte enzyme activities including enolase, pyruvate kinase, and ATPase were found in chronically exposed workers in conjunction with slightly increased fluoride levels in the body (Guminska and Sterkowicz 1975). These alterations may explain the decreased red blood cell counts observed in other studies (Hillman et al. 1979; Susheela and Jain 1983). However, none of these enzyme alterations are specific to fluoride exposure. No information is available regarding how long these effects last after the last exposure. The enzymatic effects were measured within a few hours of a single fluoride treatment, while the red blood cell effects were seen as a result of chronic exposure.

There is evidence that in patients with skeletal diseases the proportion of dialyzable and nondialyzable hydroxyproline peptides serves as an index of bone collagen turnover. A decreased proportion of nondialyzable hydroxyproline peptides in the urine of fluorosis patients indicates either a decreased rate of synthesis of new collagen or an increased utilization of newly formed collagen for matrix formation. This marker offers potential for an early, although nonspecific, indication of altered bone metabolism after long-term fluoride exposure (Anasuya and Narasinga Rao 1974). No information is available regarding how long this lasts after chronic exposure. Sudden hyperkalemia and hypocalcemia are effects seen with fluoride intoxication due to the marked potassium efflux from intact cells caused by fluoride (McIvor et al. 1985). These ionic shifts are the only serologic marker of effect that have been identified, and these changes are not unique to fluoride. They last for a few hours after exposure. Polydypsia and polyuria are also nonspecific markers of effect.

3.9 INTERACTIONS WITH OTHER CHEMICALS

The absorption of fluoride from the gastrointestinal tract of humans and/or animals is affected by the presence of several minerals including calcium, magnesium, phosphorus, and aluminum (Rao 1984). These effects are discussed in Section 3.11. No reliable data on interactions that exacerbate negative effects of fluoride were located.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to fluorine, hydrogen fluoride, or fluoride than will most persons exposed to the same level of fluorine, hydrogen fluoride, or fluoride in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of fluorine, hydrogen fluoride, or fluoride, or compromised function of organs affected by fluorine, hydrogen fluoride, or fluoride. Populations who are at greater risk due to their unusually high exposure to fluorine, hydrogen fluoride, or fluoride are discussed in Section 6.7, Populations With Potentially High Exposures.

Existing data indicate that subsets of the population may be unusually susceptible to the toxic effects of fluoride and its compounds. These populations include the elderly, people with deficiencies of calcium, magnesium, and/or vitamin C, and people with cardiovascular and kidney problems. However, these effects would not be expected at typical exposure levels (1 ppm fluoride).

A study by Spencer et al. (1980a) provides suggestive evidence that individuals with chronic renal failure may have increased fluoride retention. In eight patients with chronic renal failure on a low protein, low calcium, and low phosphorus diet, 65% of ingested fluoride was retained, compared to 20% in normal subjects. It is not known if the normal subjects were given the same diet; if the normal subjects were not on the same restrictive diet, then diet may have contributed to the observed difference in fluoride retention. The higher retention of fluoride was primarily due to the significantly decreased fluoride excretion in the renal patients. Urinary fluoride excretion was directly correlated with creatinine clearance. In mild renal failure (creatinine clearance of 50 mL/minute), urinary fluoride levels were in the low normal range (2.6 mg/day) compared to urinary fluoride levels of 2.7–4.3 mg/day with normal creatinine clearance (90–120 mL/minute). Although these data provide suggestive evidence that individuals with chronic renal failure may be unusually susceptible to the toxicity of fluoride, these data are not conclusive and it is not known if diet influenced fluoride retention and studies examining the potential toxicity of fluoride in chronic renal failure patients were not identified.

Poor nutrition increases the incidence and severity of dental fluorosis (Murray and Wilson 1948; Pandit et al. 1940) and skeletal fluorosis (Pandit et al. 1940). Comparison of dietary adequacy, water fluoride levels, and the incidence of skeletal fluorosis in several villages in India suggested that vitamin C deficiency played a major role in the disease (Pandit et al. 1940). Calcium intake met minimum standards, although the source was grains and

vegetables, rather than milk, and bioavailability was not determined. Because of the role of calcium in bone formation, calcium deficiency would be expected to increase susceptibility to effects of fluoride. Calcium deficiency was found to increase bone fluoride levels in a 2-week study in rats (Guggenheim et al. 1976) but not in a 10-day study in monkeys (Reddy and Srikantia 1971). Guinea pigs administered fluoride and a low-protein diet had larger increases in bone fluoride than those given fluoride and a control diet (Parker et al. 1979). Bone changes in monkeys following fluoride treatment appear to be more marked if the diet is deficient in protein or vitamin C, but the conclusions are not definitive because of incomplete controls and small sample size (Reddy and Srikantia 1971). Inadequate dietary levels of magnesium may affect the toxic effects of fluoride. Fluoride administered to magnesium-deficient dogs prevented soft-tissue calcification but not muscle weakness and convulsions (Chiemchaisri and Philips 1963). In rats, fluoride aggravated the hypomagnesemia condition, which produced convulsive seizures. The symptoms of magnesium deficiency are similar to those produced by fluoride toxicity. This may be because of a fluoride-induced increase in the uptake of magnesium from plasma into bone.

Although the possible relationship between fluoride in drinking water and the risk of fractures has been extensively investigated, the data are inconclusive with studies finding beneficial (Madans et al. 1983; Phipps et al. 2000; Simonen and Laittenen 1985) and deleterious (Cooper et al. 1990, 1991; Danielson et al. 1992; Jacobsen et al. 1990; Kurttio et al. 1999; Sowers et al. 1986) effects or no effects (Arnala et al. 1984; Cauley et al. 1995; Kröger et al. 1994). Clinical trials of postmenopausal women with osteoporosis have found an increased risk of nonvertebral fractures following exposure to high doses of fluoride (34 mg/day) (Haguenauer et al. 2000; Riggs et al. 1990, 1994); no effect on vertebral fracture risk was found.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to fluorine, hydrogen fluoride, and fluorides. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to fluorine, hydrogen fluoride, and fluorides. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to fluorine, hydrogen fluoride, and fluorides:

Bronstein AC, Currance PL. 1988. Emergency care for hazardous materials exposure. St. Louis, MO: The C.V. Mosby Company, 113-114, 165-166.

Ellenhorn MJ, Barceloux DG. 1988. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier Science Publishing Company, Inc. 76, 83, 531-536, 873-874, 924-929.

Goldfrank LR, Flomenvaum NE, Lewin NA, et al., eds. 1990. Goldfrank's toxicologic emergencies. Norwalk, CT: Appleton & Lange, 220-221, 745, 769-779.

In all cases of acute high-level exposure to fluorine, hydrogen fluoride/hydrofluoric acid, or fluoride, the focus of mitigation is to limit further absorption and to complex or remove the free fluoride ions from the blood while maintaining the proper electrolyte balances. The majority of relevant acute high-level exposure situations for

which mitigation information is available involve dermal and/or inhalation exposure to hydrofluoric acid or gaseous hydrogen fluoride. Some information is also available regarding mitigation of chronic oral exposure to fluoride.

3.11.1 Reducing Peak Absorption Following Exposure

Fluorine. Inhalation exposure to fluorine is treated very similarly to inhalation exposure to hydrogen fluoride. The source of exposure is removed and water used to decontaminate the patient. The eyes are washed with saline if necessary, and magnesium oxide paste can be applied (Bronstein and Currance 1988; Stutz and Janusz 1988).

Hydrogen Fluoride/Hydrofluoric Acid. In cases of dermal and inhalation exposure, the exposed persons are first removed from the source of exposure, and any particles or excess liquids are removed by brushing or blotting (Bronstein and Currance 1988). Thorough irrigation with cold water or saline is then done to further limit absorption through exposed skin and eyes. Irrigation is followed by washing the affected skin with an alkaline soap and water (Bronstein and Currance 1988; Dibbell et al. 1970).

Persistent pain is an indication that large amounts of free fluoride ions remain. In such cases, magnesium oxide paste is applied or the exposed skin is soaked in cold solutions of magnesium sulfate, calcium salts, or quaternary ammonium compounds (benzalkonium chloride, benzethonium) (Browne 1974; Goldfrank et al. 1990; Haddad and Winchester 1990). However, the evolving standard of treatment for mild to moderate burns involves massaging the affected area with a penetrating calcium gluconate gel, to avoid problems with magnesium oxide precipitation (Borak et al. 1991; Browne 1974; Goldfrank et al. 1990).

Fluoride. Ingested fluoride is rapidly absorbed from the gastrointestinal tract, but calcium and magnesium salts, antacids, and milk interfere with the absorption by binding the fluoride ion and removing the residual fluoride from the esophagus (Ellenhorn and Barceloux 1988; Goldfrank et al. 1990; Haddad and Winchester 1990; Morgan 1989). Gastric lavage with solutions of calcium gluconate, calcium carbonate, calcium lactate, calcium chloride, calcium hydroxide, calcium- or magnesium-based antacid, or aluminum hydroxide gel aid in decontaminating the gastrointestinal tract due to their action in precipitating the fluoride in the gut (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Morgan 1989). No attempt is made to neutralize the acid with orally administered sodium bicarbonate, due to the resulting exothermic reaction (Bronstein and Currance 1988). Most authorities discourage emesis due to the formation of hydrofluoric acid in the stomach (Bronstein and Currance 1988; Haddad and Winchester 1990).

The absorption of fluoride from the gastrointestinal tract of humans and/or animals is affected by the presence of several minerals including calcium, magnesium, phosphorus, and aluminum (Rao 1984). With the exception of aluminum hydroxide, no studies were located regarding the therapeutic use of these materials in humans.

Humans administered aluminum hydroxide (as antacid) had a significant increase in the fecal fluoride excretion and a decrease in the urinary excretion of fluoride (Spencer and Lender 1979; Spencer et al. 1980a). These results can be explained by a reduction in gastrointestinal absorption of fluoride due to aluminum's ability to form fluoride complexes (Spencer et al. 1981).

Calcium binds with fluoride after oral exposure, which reduces the bioavailability of fluoride. In humans, calcium and/or phosphorus administration (as bone meal, cryolite, or calcium fluoride) decreased the absorption of fluoride (Machle and Largent 1943; McClure et al. 1945). In another study, added calcium had only a limited effect on the intestinal absorption of fluoride in humans (Spencer et al. 1975c, 1980b). The discrepancy between these studies may be due to differences between the absorption of fluoride in calcium fluoride or in a form that must first be homogenized, and the absorption of fluoride in the presence of added calcium.

Magnesium may decrease the intestinal absorption of fluoride because it tends to form slightly soluble complexes with fluoride (Kuhr et al. 1987). The results of human and animal studies investigating this interaction appear to differ. Several studies have found no significant effect from orally administered magnesium oxide on either fecal or urinary fluoride excretion in humans (Spencer et al. 1977a, 1977b, 1978a). Humans administered magnesium along with fluoride as therapy for osteoporosis had diminished joint pain and resorbed periarticular calcium phosphate deposits (Kuhr et al. 1987). Magnesium appeared to reduce the adverse effects of fluoride when it was used as a treatment for osteoporosis.

3.11.2 Reducing Body Burden

Hydrogen Fluoride/Hydrofluoric Acid. Hydrogen fluoride burns are characterized by intense pain and progressive tissue destruction. The damage associated with this burn occurs in two stages. The first stage is immediate tissue damage caused by a high concentration of hydrogen ions and the second is liquefaction necrosis that is caused by free fluoride ions (Seyb et al. 1995). There are a number of recommended forms of therapy; these therapies have the common goal of binding the fluoride ion and/or altering its reactivity with tissues (Dunn et al. 1992). Recommended forms of therapy include topical treatments with calcium gluconate paste, magnesium oxide paste, and iced solutions of quaternary ammonium compounds, alcohol, or magnesium sulfate and intradermal injections of either magnesium sulfate or calcium gluconate, or intraarterial injection of calcium gluconate (Dunn et al. 1992; Seyb et al. 1995). Intra-arterial infusions of calcium gluconate are often preferred to intradermal injections due to the ability of the infusions to deliver more calcium to the burn site, better distribution of calcium in the tissues, and the need for only a single injection, as opposed to an injection for every square centimeter of affected dermal tissue (Haddad and Winchester 1990). Additionally, in burns involving the hands, multiple intradermal injections pose the risk of elevating tissue pressures and forcing the removal of the nails (Ellenhorn and Barceloux 1988; Goldfrank et al. 1990). One source reports that calcium gluconate injection was successfully used in at least 96 cases without causing damage (Browne 1974).

Several studies have compared different therapies in an attempt to identify the most effective treatment. The therapeutic effects of calcium gluconate, magnesium acetate, and magnesium sulfate on hydrofluoric acid burns of shaved Sprague-Dawley rats were compared using intradermal and subcutaneous injection (Harris et al. 1981). Although this study found that injection of calcium gluconate, but not the magnesium compounds, was irritating in the absence of a burn, and the duration, depth, and area of lesions were reduced with the magnesium compounds compared with calcium gluconate, no reports were located of using intradermal injection of magnesium compounds in humans. Seyb et al. (1995) found that subcutaneous injections of 10% calcium gluconate and magnesium sulfate solution and topically applied calcium gluconate mixed with dimethyl sulfoxide significantly reduced the damage caused by hydrogen fluoride exposure in rats exposed to 70% hydrogen fluoride for 60 seconds followed by continuously rinsing with tap water for 5 minutes. Treatment with topically applied dimethyl sulfoxide only or calcium gluconate only did not affect the degree of tissue damage. In contrast, Dunn et al. (1992) found that injection of 10% calcium gluconate was the least effective therapy in pigs following topical application of 38% hydrogen fluoride. The most effective treatments were soaking in calcium acetate or iced Zephiran (benzalkonium chloride), or injection of 5% calcium gluconate.

Fluoride. A study by Khandare et al. (2000) provides suggestive evidence that co-administration of fluoride and tamarind results in increased urinary excretion of fluoride and decreased bone fluoride levels in dogs.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Hydrogen Fluoride/Hydrofluoric Acid. The primary focus of research on reducing the toxic effects following dermal exposure to hydrogen fluoride or hydrofluoric acid is on methods for reducing absorption and decreasing the amount of fluoride ions. The tissue damage associated with hydrogen fluoride exposure is believed to be caused by the binding of fluoride ions with tissue calcium and magnesium cations to form insoluble salts, which are believed to interfere with cellular metabolism, inducing cellular death and necrosis. Thus, the most effective method for interfering with the mechanism of action is removal of the fluoride ions; these methods are discussed in Section 3.11.2.

Fluoride. The major treatment strategies for long-term, low-level exposure to fluorides are removal of the source of exposure and administration of compounds that reduce intestinal absorption. Skeletal fluorosis has been reported to be partially reversed 8–15 years after the elevated exposure ended (Grandjean and Thomsen 1983). Sclerosis of the trabecular bone in ribs, vertebral bodies, and pelvis faded, but calcification of muscle insertions and ligaments was not altered. Techniques that increase bone turnover or bone resorption might be effective in reversing skeletal fluorosis. However, no information on such techniques were located.

Chinoy and associates have examined the effectiveness of calcium, ascorbic acid, vitamin E, and vitamin D in reversing the reproductive effects associated with oral exposure to sodium fluoride. Administration of ascorbic acid and/or calcium and cessation of sodium fluoride exposure enhanced the recovery of sperm function and morphology and testicular damage, as compared to no treatment, in rats (Chinoy et al. 1993), mice (Chinoy and

Sharma 2000), and rabbits (Chinoy et al. 1991). The combined administration of ascorbic acid and calcium was the most effective treatment. Postexposure administration of vitamins E and/or D was also effective in the recovery of sodium-fluoride induced testicular effects in mice (Chinoy and Sharma 1996). Likewise, posttreatment administration of ascorbic acid and/or calcium and vitamins E and/or D also aided in the recovery of ovarian effects in mice (Chinoy and Patel 1998; Chinoy et al. 1994). It is believed that the antioxidant properties of ascorbic acid and vitamin E aid in the recovery of fluoride damage. Vitamin D promotes the intestinal absorption of calcium and phosphorus, thus maintaining the optimal blood concentration of these elements (Chinoy and Patel 1998). The calcium may act by forming insoluble complexes with fluoride (Chinoy and Patel 1998; Chinoy et al. 1994).

3.12 ADEQUACY OF THE DATABASE

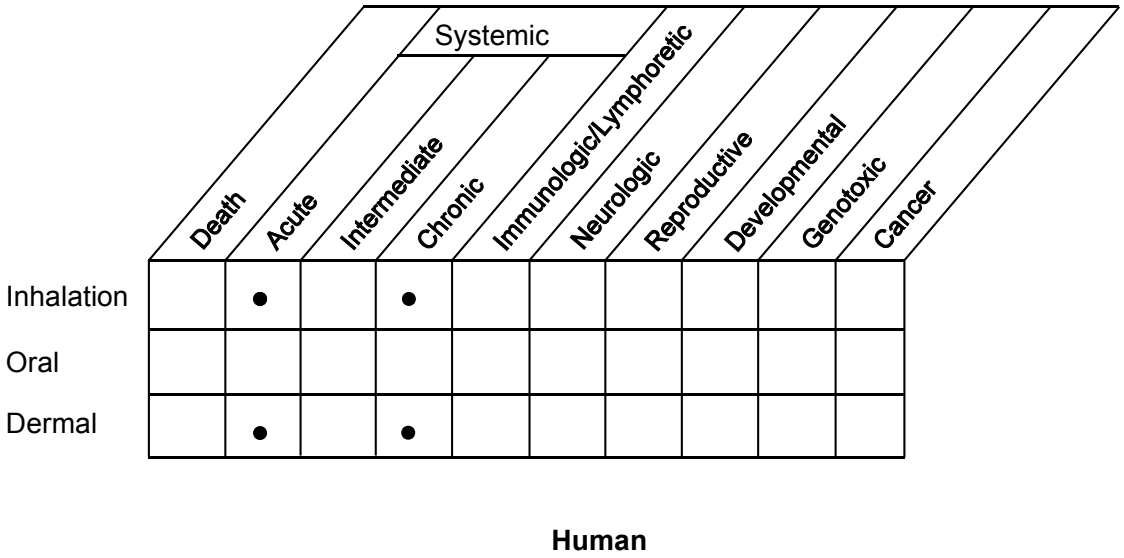
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of fluorine, hydrogen fluoride, or fluoride is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of fluorine, hydrogen fluoride, or fluoride.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

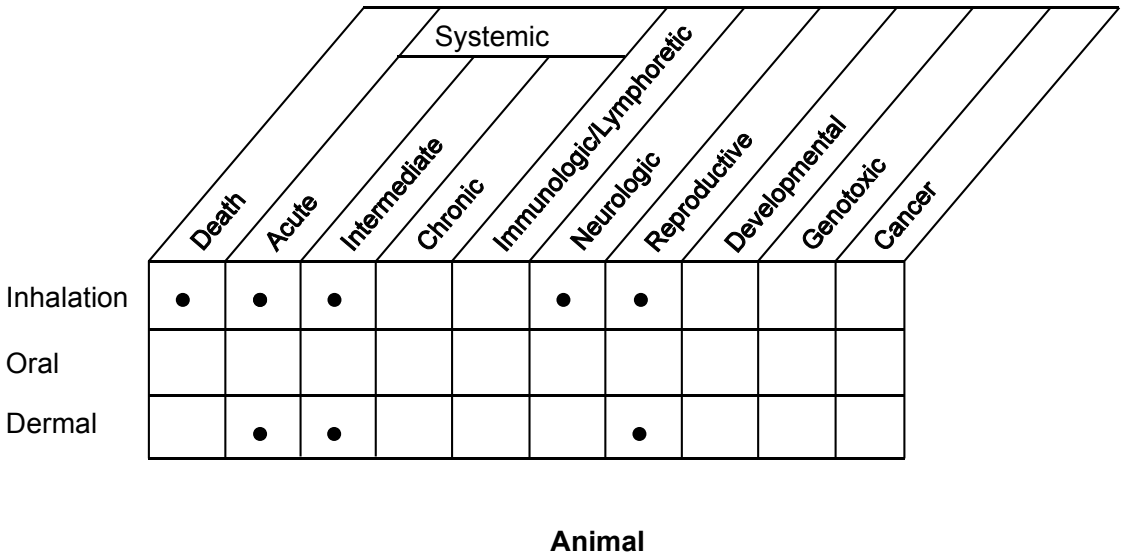
3.12.1 Existing Information on Health Effects of Fluorine, Hydrogen Fluoride, and Fluorides

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to fluorine, hydrogen fluoride, or fluoride are summarized in Figures 3-5, 3-6, and 3-7, respectively. The purpose of these figures are to illustrate the existing information concerning the health effects of fluorine, hydrogen fluoride, or fluoride. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to

Figure 3-5. Existing Information on Health Effects of Fluorine



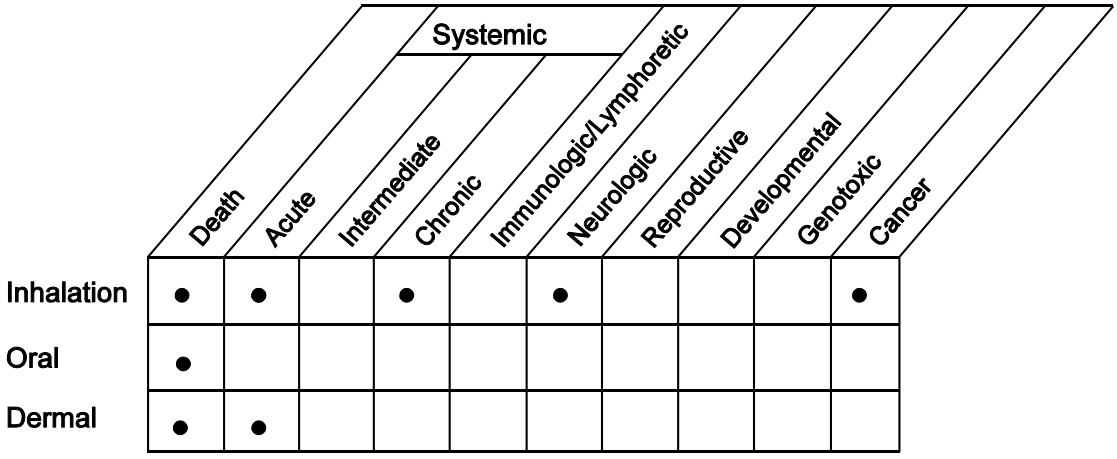
Human



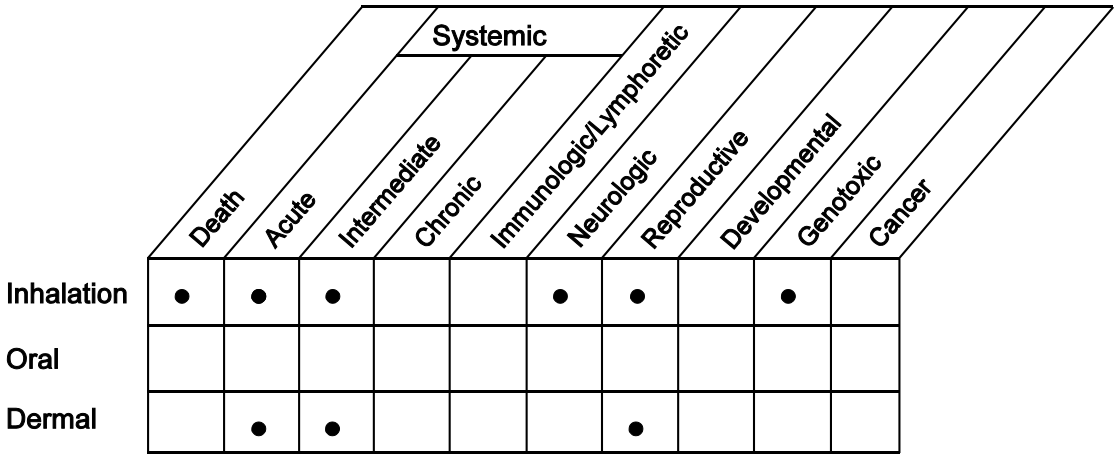
Animal

- Existing Studies

Figure 3-6. Existing Information on Health Effects of Hydrogen Fluoride/ Hydrofluoric Acid



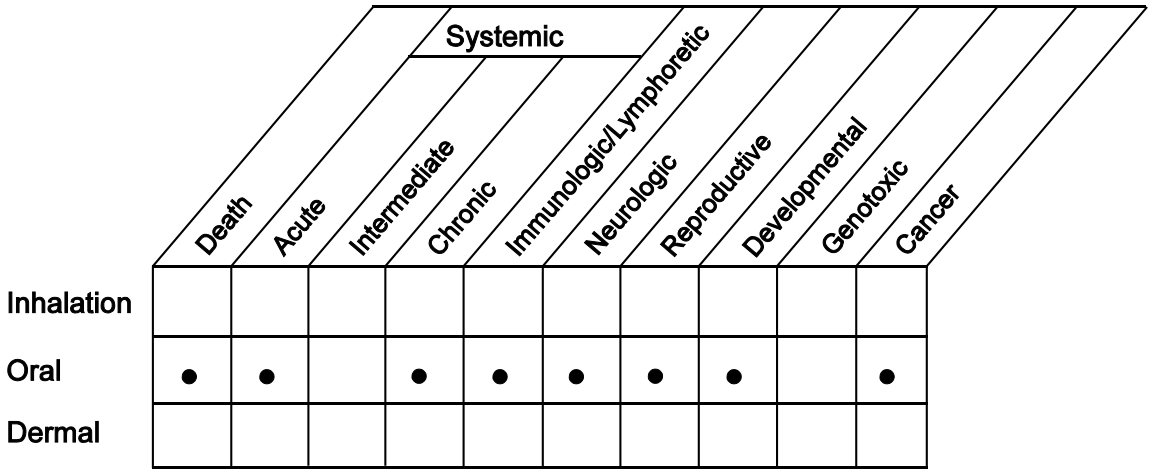
Human



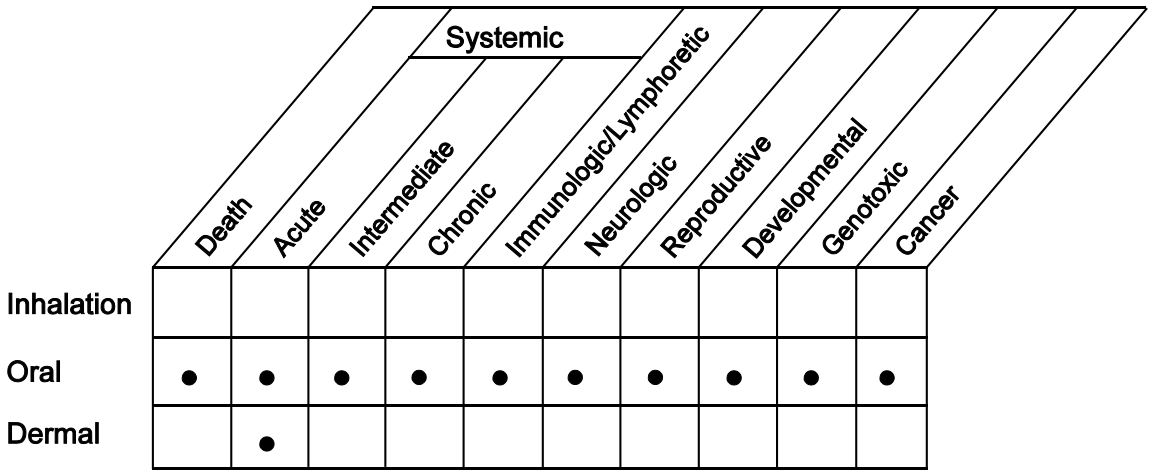
Animal

● Existing Studies

Figure 3-7. Existing Information on Health Effects of Fluoride



Human



Animal

● Existing Studies

conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

There are many case reports and epidemiological studies investigating the health effects of hydrogen fluoride in humans by the inhalation and dermal routes, and the health effects of fluoride compounds by the inhalation and oral routes. There are also limited data from experimental human exposure to fluorine. Most human studies of the health effects of oral exposure to fluoride are case reports of acute and chronic oral exposure to sodium fluoride, and human studies of the health effects of dermal exposure to fluorides are generally case reports of acute dermal exposure to hydrofluoric acid.

Human fatalities have resulted from both oral exposure to sodium fluoride and dermal exposure to hydrofluoric acid. Dermal exposure to hydrofluoric acid is often accompanied by inhalation of hydrofluoric acid fumes. Human studies and case reports have investigated the effects of nonlethal oral doses of sodium fluoride, although only after acute exposure. These exposures have resulted in mostly gastrointestinal effects and consequences of hypocalcemia (e.g., nervous system and cardiovascular effects). Exposure to fluorine gas causes respiratory, ocular, and dermal irritation in humans after acute exposure. One study on chronic exposure to fluorine was located. Chronic human studies have generally examined health effects in workers exposed to hydrogen fluoride or fluoride-containing dusts by inhalation, and populations exposed to ionic fluoride through drinking water. These studies have investigated the relationship between fluoride and neurological and reproductive effects and cancer.

Studies conducted on animals have been fairly extensive, and have focused on the health effects following inhalation of hydrogen fluoride and oral exposure to fluoride. A few studies on inhalation exposure to fluorine also exist. Dermal studies in animals are limited to those investigating dermal and ocular effects from exposure to fluorine, hydrofluoric acid, and sodium fluoride. A number of studies on the genotoxicity of fluoride were located.

3.12.2 Identification of Data Needs

The following section will discuss data needs by category and by chemical (fluorine, hydrogen fluoride, and fluorides). Although the toxicological data on fluorine are severely limited, such data are not needed, since fluorine is so reactive that human exposure at hazardous waste sites is unlikely.

Acute-Duration Exposure. Inhalation of fluorine can cause respiratory irritation, dyspnea, and death (Keplinger and Suissa 1968; Stokinger 1949). Inhalation of concentrated hydrofluoric acid fumes can cause pulmonary edema, hyperkalemia, hypocalcemia, and death (Chela et al. 1989; Kleinfeld 1965; Tepperman 1980). Acute dermal exposure to hydrofluoric acid will cause burns (Chela et al. 1989; Kleinfeld 1965; Mullett et al. 1987; Tepperman 1980). Gastrointestinal upset (Hoffman et al. 1980; Rao et al. 1969), cardiovascular disturbances, or death can result from accidental consumption of large amounts of sodium fluoride or other soluble fluoride salts (Eichler et al. 1982; Sharkey and Simpson 1933). The toxicity and pharmacokinetic data at the present time are not sufficient to derive acute duration MRLs for inhalation of fluorine or hydrogen fluoride or oral exposure to sodium fluoride because the exposure data in humans are not well quantified. Regarding acute oral toxicity, it should be mentioned that because the rat does not vomit, this would not be an appropriate model to use to determine levels of exposure that cause gastrointestinal distress. In addition, there is no way of determining if the animal is suffering from gastrointestinal discomfort. Further information concerning the levels of oral exposure to sodium fluoride, calcium fluoride, or hydrofluoric acid that cause acute effects in humans such as gastrointestinal distress would be useful because there are populations surrounding hazardous waste sites that might be exposed to these forms of fluoride for brief periods. Inhalation of fluorine can cause respiratory irritation, dyspnea, and death (Keplinger and Suissa 1968; Stokinger 1949). Inhalation of concentrated hydrofluoric acid fumes can cause pulmonary edema, hyperkalemia, hypocalcemia, and death (Chela et al. 1989; Kleinfeld 1965; Tepperman 1980). Acute dermal exposure to hydrofluoric acid will cause burns (Chela et al. 1989; Kleinfeld 1965; Mullett et al. 1987; Tepperman 1980). Gastrointestinal upset (Hoffman et al. 1980; Rao et al. 1969), cardiovascular disturbances, or death can result from accidental consumption of large amounts of sodium fluoride or other soluble fluoride salts (Eichler et al. 1982; Sharkey and Simpson 1933). The available data were sufficient to derive acute-duration inhalation MRLs for fluorine and hydrogen fluoride. The data were inadequate for derivation of an acute-duration oral MRL for fluoride. Further information concerning the levels of oral exposure to sodium fluoride, calcium fluoride, or hydrofluoric acid that cause acute effects in humans such as gastrointestinal distress would be useful because there are populations surrounding hazardous waste sites that might be exposed to these forms of fluoride for brief periods.

Intermediate-Duration Exposure. There are limited data on the toxicity of fluorine following intermediate-duration exposure; pulmonary effects were observed in rats, dogs, and rabbits (Stokinger 1949). The data were not considered sufficient for derivation of an intermediate-duration inhalation MRL for fluorine. The limited database for hydrogen fluoride also suggests that the respiratory tract is a sensitive target. Nasal irritation has been observed in humans (Largent 1960) and pulmonary effects have been observed in animals (Stokinger 1949). The database for hydrogen fluoride was considered adequate for derivation of an intermediate-duration inhalation MRL. Bone and tooth fluoride levels were elevated, suggesting that these could also be target organs for intermediate exposure to fluorine or hydrogen

fluoride. One might also expect that the musculoskeletal system may be a target of intermediate-duration oral exposure to fluoride. Studies in mice (Greenberg 1986; NTP 1990) suggest that the kidney may also be a target of near-lethal fluoride levels, but there are no data from such high exposures in humans. The toxicity and pharmacokinetic data at the present time are not sufficient to derive an intermediate-duration oral MRL for fluoride. Studies examining the long-term toxicity of fluorine and ingested fluoride are needed for derivation of intermediate-duration MRLs.

Chronic-Duration Exposure and Cancer. Small amounts of ionic fluoride given chronically in the drinking water are recognized as being beneficial to human teeth (DHHS 1991). The mechanisms of action include incorporation of fluoride into enamel preeruptively, inhibition of demineralization, enhancement of remineralization, and inhibition of bacterial activity in dental plaque (DHHS 2000). Chronic exposure of children to excessive amounts of fluoride can result in mottled teeth (fluorosis) (Hodge and Smith 1972; Mann et al. 1987), but variations in methods of reporting dental fluorosis make it difficult to thoroughly compare studies. The development of a method for quantitating dental fluorosis that is sensitive, specific, reliable, and acceptable to the public would help in determining the fluoride doses leading to varying degrees of fluorosis. Epidemiological evidence exists that the incidence and severity of dental fluorosis has increased in the United States (DHHS 1991; Heifetz et al. 1988). Further examination of the etiology and trends in prevalence may be useful. Chronic inhalation exposure to high levels of hydrogen fluoride and fluoride dusts, or chronic oral exposure to high doses of fluoride can cause skeletal deformities and joint pain (Bruns and Tytle 1988; Goldman et al. 1971; Fisher et al. 1981; Kemp et al. 1942; Leone et al. 1955; Moller and Gudjonsson 1932; Pandit et al. 1940; Sauerbrunn et al. 1965; Singh et al. 1963). Some data from case studies are available regarding nutritional states that exacerbate fluorosis (Kemp et al. 1942; Pandit et al. 1940). Epidemiological studies addressing the effect of nutrition on the prevalence and severity of dental and skeletal fluorosis may be useful. Numerous epidemiology studies have examined the possible association between consumption of fluoride in drinking water and the risk of fractures (Arnala et al. 1986; Cauley et al. 1995; Cooper et al. 1990, 1991; Danielson et al. 1992; Jacobsen et al. 1990; Kröger et al. 1994; Kurttio et al. 1999; Madans et al. 1983; Phipps and Burt 1990; Phipps et al. 2000; Simonen and Laittinen 1985; Sowers et al. 1986); these studies provide conflicting results and limitations of the study design preclude establishing a causal relationship. Clinical studies of women with osteoporosis treated with fluoride provide evidence that ingesting high doses of fluoride can result in an increase in nonvertebral fracture risk (Haguenauer et al. 2000; Riggs et al. 1990, 1994). The Riggs et al. (1990, 1994) study was used as the basis of a chronic-duration oral MRL for fluoride. Analytical epidemiological studies of the association, if any, between bone fractures and factors such as fluoride intake, fluoride blood levels, diet, and body levels of nutrients such as calcium may be useful. Target organs other than bones and teeth for chronic exposure to fluoride for humans are not known. There is some evidence of hepatic (Greenberg 1982a) and renal (Daston et al.

1985; Kessabi et al. 1985) effects of fluoride in animals, but minimal information regarding possible effects in humans. Additional studies specifically addressing effects on these systems may be useful.

The osteosarcoma rate in males living in fluoridated areas has increased markedly in recent years, but thorough statistical analyses concluded that the effect is not due to fluoridation (Hoover et al. 1991). Nonetheless, analytical epidemiology studies to determine the risk factors for osteosarcoma may be useful. Such studies should include analysis of fluoride exposure and bone levels of fluoride. Case-control studies of people with osteosarcoma could be particularly useful. The NTP oral carcinogenicity study for sodium fluoride concluded that there is equivocal evidence that fluoride is a carcinogen in male rats, but not in female rats or mice of either gender (NTP 1990). Higher doses may have been attainable in female rats and mice of both genders. Another rat carcinogenicity study found no evidence that fluoride is a carcinogen (Maurer et al. 1990), but was limited in several different aspects. Additional animal cancer bioassays may be useful in addressing this issue. Additional systemic effects may be understood after further investigation.

The existing data do not demonstrate that fluoride is a human carcinogen. The epidemiological studies indicate that a carcinogenic effect of fluoride is not likely to be a health risk. Evidence of genotoxicity was seen in *in vitro* studies at very high concentrations of fluoride. It is questionable whether these findings are relevant to humans (Caspary et al. 1987, 1988; NTP 1990).

Genotoxicity. There is a significant database on the genotoxicity of fluoride compounds in several species and several cell types. However, the results from well-characterized systems are much more limited and additional well-designed experiments would be useful in resolving contradictory data. The results have been inconsistent in many instances, but a consensus is developing that at toxic levels (>10 µg/mL, and usually seen at >40 µg/mL), there may be a general inhibition of enzymes, including the DNA polymerases (Caspary et al. 1987, 1988). While sodium fluoride may not be directly reactive with DNA, biochemical studies would be useful for establishing a mechanism for the cellular toxicity seen at high doses of fluoride compounds.

Reproductive Toxicity. The reproductive toxicity of fluoride has been assessed in animals following inhalation exposure to fluorine and hydrogen fluoride and in humans and animals following oral exposure to fluoride compounds. No dermal reproductive toxicity data were identified. Reproductive toxicity data following inhalation exposure are limited to a report of testicular degeneration in rats exposed to a high concentration of fluorine gas (Stokinger 1949); and in dogs exposed to hydrogen fluoride (Stokinger 1949). Some reproductive effects have been observed in humans consuming drinking water with high levels of fluoride. A decrease in fertility was found in women living in communities with high fluoride levels in municipal water, as compared to women living in areas with low fluoride levels (Freni et al.

1994). Another study found decreased serum testosterone levels in men with skeletal fluorosis and in men consuming water with high levels of fluoride (Susheela and Jethanandani 1996). Although some studies have reported an increased incidence of Down's syndrome among populations exposed to high levels of fluoride, these studies have been refuted (Berry 1958; Erickson et al. 1976; Needleman et al. 1974). The available human studies have limited value in assessing the reproductive potential of fluoride. A number of animal studies provide evidence that the reproductive system is a target of fluoride toxicity at high exposure levels. The observed reproductive effects include decreases in serum testosterone levels in rats (Araibi et al. 1989; Narayana and Chinoy 1994), testicular damage in rats (Araibi et al. 1989; Krasowska and Wlostowski 1992; Susheela and Kumar 1991), and alterations in spermatogenesis or sperm morphology in rats (Araibi et al. 1989; Chinoy et al. 1992, 1995), mice (Chinoy and Sequeira 1992), rabbits (Kumar and Susheela 1994, 1995; Susheela and Kumar 1991), and guinea pigs (Chinoy et al. 1997). Mating the exposed males with unexposed females resulted in decreased fertility (Chinoy and Sequeira 1992; Chinoy et al. 1992). However, other studies have not found alterations in testosterone levels (Sprando et al. 1997), testicular histopathology (Sprando et al. 1998), or in sperm (Dunipace et al. 1989; Li et al. 1987a) in rats or mice exposed to similar concentrations of sodium fluoride. Reproductive effects have also been observed in females. Reduced fertility was observed in female mice (Messer et al. 1973); a similarly designed study did not support these results (Tao and Suttie 1976). Additional support for an adverse effect of high fluoride levels on reproduction comes from studies in dogs and birds (Guenter and Hahn 1986; Hoffman et al. 1985; Shellenberg et al. 1990; Van Rensburg and de Vos 1966). The available human and animal data provide suggestive evidence that the reproductive system is a target of fluoride toxicity at high exposure levels; however, additional studies are needed to resolve the apparent conflicting results. An oral multigeneration study would be useful to establish dose-response relationships; inhalation and dermal exposure studies would be useful for determining whether the reproductive system is also a target of toxicity following these routes of exposure.

Developmental Toxicity. There are no studies in humans or animals regarding the developmental effects of inhaled hydrogen fluoride or following dermal exposure to fluoride compounds. Several human and animal studies have examined the developmental toxicity of fluoride following oral exposure. No effect on the incidence of birth defects were found in the children of women drinking fluoridated water (Erickson et al. 1976). Other epidemiology studies have suggested that exposure to high levels of fluoride in water can result in developmental effects in humans. An increase in the occurrence of spina bifida was found in children living in area of India with high fluoride levels in water (4.5–8.5 ppm) (Gupta et al. 1995). A decrease in intelligence, as measured by IQ scores, was found in children exposed to high levels of fluoride in the water (Li et al. 1995a; Lu et al. 2000). However, as noted previously, the Gupta et al. (1995), Li et al. (1995a), and Lu et al. (2000) studies appear to have considerable study design inadequacies. In general, studies in conventional laboratory species have not found developmental effects in rats exposed to 12.26–21 mg fluoride/kg/day (Collins et al. 1995; Heindel et al. 1996; Ream et

al. 1983) or rabbits exposed to 13.21 mg fluoride/kg/day (Heindel et al. 1996). A two-generation study in rats did not find developmental effects in the first generation offspring of dams exposed 23 mg/kg/day, but an increased number (statistical analysis was not performed) of abnormal pups and affected litters was seen the second generation (Marks et al. 1984). Adverse developmental effects of oral fluoride exposure have also been observed in calves (Krook and Maylin 1979; Maylin and Krook 1982) and mink (Aulerich et al. 1987). Additional developmental toxicity studies are needed to assess whether the developing organism is a sensitive target of fluoride toxicity. These studies should assess multiple routes of exposure, involve multigenerational exposure, and evaluate the potentially sensitive developing nervous system.

Immunotoxicity. A review of human studies has shown that fluoride in drinking water has no adverse effects on immunologically mediated reactions or allergies (Austen et al. 1971). This suggests that the immune system is not a sensitive target for fluoride toxicity following oral exposure. Additional information is probably not needed at this time.

Neurotoxicity. Because fluoride interacts with calcium ions needed for effective neurotransmission, fluoride can affect the nervous system. However, while acute effects on the nervous system have been observed in humans, it is not known whether chronic exposure to hydrogen fluoride or fluoride can cause nervous system effects. Human and animal studies have shown minor changes in neurological function after inhalation exposure to hydrogen fluoride. Overt signs or behavioral signs of neurotoxicity were not noted, except for alterations in conditioned responses and evidence of depression observed in rats (Sadilova et al. 1965). Further neurological testing may be warranted to ascertain the conditions involved and the extent to which the nervous system is a target organ for fluoride toxicity.

Epidemiological and Human Dosimetry Studies. Since fluoride is available in the drinking water in many communities, many epidemiological studies have been conducted regarding its health effects. Epidemiological studies of people who have been exposed to hydrogen fluoride and other fluoride compounds occupationally have also been performed (Chan-Yeung et al. 1983a, 1983b; Czerwinski et al. 1988; Kaltreider et al. 1972). Because of the wide use of fluoride in industry and dental hygiene, it is likely that subpopulations vary in their level of exposure to fluoride. Human dosimetry studies exist that indicate that fluoride levels in the urine can be used as an indication of recent exposure (Carlson et al. 1960a; Collings et al. 1952; Machle and Largent 1943). Additional studies correlating environmental measurements of fluoride with urinary excretion data and health effects would be useful for establishing a dose response for health effects in humans.

Biomarkers of Exposure and Effect.

Exposure. The level of fluoride in urine is the best biomarker of acute exposure (Ekstrand et al. 1983; Hodge and Smith 1977). However, because chronic exposure to fluoride results in sequestration in bone, levels in the urine cannot be correlated with levels of chronic exposure (Carlson et al. 1960a). This biomarker is specific for acute and intermediate duration exposure to this chemical.

Effect. The most sensitive biomarkers of effect for fluoride are alterations in teeth and bones following chronic exposure (Knaus et al. 1976). Of these, tooth alterations are more sensitive, but they occur only during childhood (DHHS 1991; Heifetz et al. 1988). Changes in glucose or lipid metabolism (Dousset et al. 1984; Shearer 1974; Watanabe et al. 1975), and in erythrocyte enzyme activities (Guminska and Sterkowicz 1975) have been noted following acute exposure. Specific biomarkers of effects following acute exposures have not been well identified, and would be useful in monitoring short-term effects, such as might be expected to occur in hazardous waste site workers.

Absorption, Distribution, Metabolism, and Excretion. Data exist that indicate that a high percentage of hydrogen fluoride is rapidly absorbed following acute inhalation exposure (Collings et al. 1952; Rye 1961). Rates of absorption differ between human studies, because the reported times to peak urinary fluoride levels are different (2–4 vs. 10 hours) (Collings et al. 1952; Rye 1961). One human study reports absorption of fluoride from acute inhalation of rock phosphate dust, with time to peak urinary fluoride of 10 hours, and similar excretion kinetics to that found following hydrogen fluoride inhalation (Rye 1961). One acute animal study described the rate and extent of absorption following inhalation exposure to hydrofluoric acid (Morris and Smith 1982). Data on chronic absorption, extent of absorption, and potential for saturation were not located, but would be useful for predicting potential effects in persons exposed to hydrogen fluoride or fluoride dusts at low levels over extended periods of time.

Soluble fluoride is rapidly and almost completely absorbed following oral exposure of humans or animals (Armstrong et al. 1970; Carlson et al. 1960a; Ekstrand et al. 1977b, 1983; Ericsson 1958; McClure et al. 1945; Whitford and Pashley 1984; Zipkin and Likins 1957). However, the degree of absorption is affected by a number of other factors (Rao 1984).

Although dermal absorption has not been studied per se, toxicity following acute dermal exposure to hydrofluoric acid (e.g., hypocalcemia) provide adequate evidence that this is a significant route of exposure (Browne 1974; Dale 1951; Dibbell et al. 1970). However, it should be noted that in some cases, the effects reported for dermal exposure may have been caused by inhalation of hydrofluoric acid fumes as well as injury to skin. The existing data on dermal exposure to sodium fluoride (Essman et al. 1981)

are not sufficient to determine absorption. Because hydrofluoric acid readily dissolves in water and reacts readily with a number of compounds and metals, contamination of water or the ground would result in the formation of fluoride salts. Therefore, populations surrounding hazardous waste sites would be more likely to be exposed dermally to fluoride salts than to hydrofluoric acid. However, cleanup workers or members of the public who came into contact with leaking drums could be dermally exposed to hydrofluoric acid. Additional animal studies regarding rate and extent of absorption following dermal exposure would be useful for clarifying the effects seen following dermal or inhalation exposure.

The development of systemic effects following whole body exposure to fluorine indicates that fluorine is absorbed (Stokinger 1949). The rate and extent of absorption are not known.

Regardless of the route of administration, fluoride is found in the plasma (Morris and Smith 1983; Perkinson et al. 1955), and accumulates in bones and teeth. Fluoride can accumulate in the kidney (Whitford and Taves 1973) and aorta (Smith et al. 1960). Further information concerning distribution would be useful to determine if there are target organs of fluoride exposure in addition to the skeletal, gastrointestinal, and cardiovascular systems. In addition, while it is known that elevated bone fluoride levels decrease with time if the exposure source is removed, more information about the kinetics of this process would be useful.

Fluoride interacts with other elements, particularly in bone formation (McCann and Bullock 1957; Neuman et al. 1950). The ion is also known to interact with enzymes in the body (Capozzi et al. 1967; Cimasoni 1966; Halton et al. 1984; Smith et al. 1959). Although there are extensive data on *in vitro* inhibition of enzymes, very few data exist regarding the biological significance of these interactions. These *in vitro* studies were carried out using concentrations that exceed body fluid concentrations by a factor greater than 100. Several glycolytic enzymes are inhibited at fluoride concentrations of 38 ppm (Capozzi et al. 1967). A few enzymes have been identified that are inhibited at *in vitro* concentrations of <10 ppm (Cimasoni 1966; Smith et al. 1959). Further information on the biological significance of these enzyme reactions would be useful for assessing the mechanisms by which fluoride affects human health.

The excretion of fluoride in the urine of humans following inhalation or oral exposure is well characterized in its relationship to recent fluoride exposure (Ekstrand et al. 1983; Hodge and Smith 1977). However, while it is known that bone fluoride concentration increases with age (Smith et al. 1953), the total steady-state excretion level when people are chronically exposed to low-levels of fluoride is not well characterized. Reliable data are also lacking regarding the contribution of sweat to fluoride excretion. Data are lacking concerning excretion following dermal exposure; however, there is no evidence to suggest that excretion following dermal exposure would differ from that following oral or inhalation exposure.

Comparative Toxicokinetics. Because fluoride is generally present in the drinking water, abundant human data exist concerning the kinetics of fluoride in humans. Fewer data were located for animals that are considered to be appropriate models for humans. Human and animal data exist that indicate that the most likely target organs (bones and teeth) are similar across species for intermediate- and chronic-duration exposures (Derryberry et al. 1963; Machle and Scott 1935; Wagner et al. 1958). However, as mentioned before, the lack of a vomit reflex in rats may preclude their use as an animal model for acute oral exposure to fluoride compounds.

There is good evidence that there are marked species and strain differences regarding tolerance to increased levels of oral fluoride. For example, beef and dairy heifers show susceptibility to levels as much as 100 times lower than those causing some degree of pathology in laying and breeding hens (Suttie 1980). Whitford et al. (1991) compared the major features of fluoride pharmacokinetics (the plasma, renal, and skeletal clearances) in adult dogs, cats, rabbits, rats, and hamsters. While the clearances among the species were qualitatively similar, the dog most closely resembled pharmacokinetics in humans.

Methods for Reducing Toxic Effects. Methods have been published for limiting oral and dermal absorption of fluoride compounds (Bronstein and Currance 1988; Goldfrank et al. 1990; Haddad and Winchester 1990) and for counteracting the hypocalcemia, hypomagnesemia, and hyperkalemia that are produced by fluoride in acute high-level exposure situations (Abukurah et al. 1972; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Although there is a report in an animal model that intradermal injection of magnesium acetate or magnesium sulfate is more effective than injection of calcium gluconate (Harris et al. 1981), a report of human case studies found that the calcium gluconate method was 100% effective and did not cause tissue damage (Browne 1974). Therefore, it is not clear whether intradermal injection of magnesium acetate or magnesium sulfate should be explored. The only information located on treatment strategies for long-term exposures to excessive amounts of fluorides involved reducing exposure either by removing the source or reducing absorption. Fluoride excretion can be increased by the administration of aluminum hydroxide as antacid (Spencer et al. 1980a). Other studies show that fluoride absorption is decreased in the presence of calcium (Machle and Largent 1943; McClure et al. 1945), especially in combination with carbonate (Jowsey and Riggs 1978), although these studies were not designed to develop treatment strategies. Strategies for increasing bone turnover might also be useful, but no investigations of such methods were located. Research on using dietary supplements or increasing bone turnover for mitigating adverse effects of chronic exposure to fluoride would be helpful, especially in the case of chronic exposure to drinking water that has been contaminated with fluoride.

Children's Susceptibility. The available human studies that examined the toxicity of fluoride in children primarily focused on the skeletal system. The studies showed that exposure to moderate levels of fluoride can result in dental fluorosis. However, the effect is widely considered a cosmetic rather than function effect. Because more fluoride is deposited in children's bones than adults, there is a need for additional studies to assess whether children are more susceptible to skeletal effects. Human studies have suggested that high doses of fluoride may result in spina bifida (Gupta et al. 1995) or decreased intelligence (Li et al. 1995a; Lu et al. 2000) but, as noted previously, the Gupta et al. (1995), Li et al. (1995a), and Lu et al. (2000) studies appear to have major study design deficiencies. In general, animal studies have not found developmental effects following oral exposure; however, these studies did not examine neurodevelopmental end points that may be a sensitive target. Additional animal studies are needed to assess neurodevelopmental potential of fluoride.

In general, the available toxicokinetic data did not examine potential differences between adults and children; toxicokinetic studies examining how aging can influence absorption rates would be useful in assessing children's susceptibility to fluoride toxicity.

Child health data needs relating to exposure are discussed in Section 6.8.1 Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Ongoing studies pertaining to fluorine, hydrogen fluoride, or fluoride have been identified and are shown in Table 3-9.

Table 3-9. Ongoing Studies on the Health Effects of Fluoride

Investigator	Affiliation	Research description
Ziegler, EE	University of Iowa	Toxicokinetic properties in infants
Levy, SM	University of Iowa	Bone development in children
Boskey, AL	Hospital for Special Therapies	Osteoporosis therapy